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THE PREPARATION AND PROPERTIES OF A MODIFIED GELATIN (OXYPOLYGELATIN) AS AN ONCOTIC SUBSTITUTE FOR SERUM ALBUMIN*

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PART I. PREPARATION AND CHEMICAL PROPERTIES

1. *Introduction*

During the first part of the last war, before the success of the blood plasma program had been assured, an intense search was made for substances that could be used for transfusion in place of human plasma. The most widely investigated substitutes up to that time had been gum acacia, pectins, and blood proteins from lower animals. The first two were not acceptable because of possibility of tissue damage due to prolonged storage arising from the inability of the body to digest these complex polysaccharides. The last was objectionable because of the antigenicity of the foreign serum proteins and the consequent danger of allergic reaction upon repeated injections. Since attempts to deantigenize[1] proteins without loss of solubility or other necessary properties had not been completely successful, a search was made for a suitable nonantigenic material. The properties essential for a satisfactory plasma substitute are the following:

1. It should be bland and should be well tolerated in pathological as well as normal physiological states, and should

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[1]The authors have found no precedent for the use of this word, which is used here in place of "despeciation," which implies a loss of native specificity without ruling out the possible acquisition of new specificities, or ability to induce antibody formation. To "deantigenize" means to destroy the ability to induce antibody formation or hypersensitivity.

not interfere with the processes of tissue repair or the defense mechanisms against disease.

2. It should be incapable of inducing antibody formation (or a state of hypersensitivity) or of producing allergic reactions in hypersensitive individuals.
3. It should be of sufficiently great molecular size to be retained in the circulation for a period of several hours (we arbitrarily selected a retention value of 25 to 50 percent for 5 hours in hemorrhaged rabbits).
4. Its oncotic pressure should be that of serum albumin.
5. It should be digestible and easily cleared from the tissues.
6. It should be economical to prepare and be available in practical amounts.
7. It should be stable under a variety of conditions, including sterilization by heat, and be easy to administer in emergencies and under field conditions.
8. It should be easily reproducible and subject to standardization.

Of all the substances considered, gelatin seemed to offer the most promise, since it is non-antigenic, if carefully prepared, and is readily digested by proteolytic enzymes present in the body tissues. The chief objection to gelatin is its tendency to gel at temperatures practical for injection, and when the gelling power is decreased by prolonged heating, to form "degraded gelatin," the molecular size is so diminished that rapid leakage from the circulation occurs and its value as an oncotic agent is greatly reduced.

We subjected gelatin to treatment with a wide variety of reagents in the attempt to reduce its gelling property and at the same time to maintain a practical molecular size and osmotic pressure. Special attention was given to reagents which would cause coupling of gelatin molecules into larger complexes and would increase the number of groups ionizable at *p*H 7.3.

The reactions which were found to give the best product were the condensation of gelatin with glyoxal and subsequent oxidation with hydrogen peroxide. Because of the chemical complexity and heterogeneity of gelatin much remains to be learned about the detailed chemistry of these reactions; however, enough was learned to permit empirical standardization and control of preparations and to justify some conclusions

concerning the nature of the reactions and their influence on the physiological behavior of the product.

Since it would be impractical to describe in detail all of the investigation, covering a period of about five years, we have selected those data which are of greatest value to the problem. Further details may be obtained from our Final Report of Contract OEMcmr-153.

In reports to the Committee on Medical Research the modified gelatin was designated as "Oxypolygelatin." Although the increase in molecular size of gelatin was brought about by a condensation reaction with glyoxal rather than by a polymerization, the term "polygelatin" was used for convenience, and the name OXYPOLYGELATIN (OPG) has been assigned to the product obtained by oxidation of the glyoxal-treated gelatin.

2. *Preparation of Oxypolygelatin*

After preliminary studies had been made with several gelatins, the major developmental work was carried out with a special ossein gelatin (C-1) supplied by Dr. D. Tourtellotte of the Knox Gelatine Company. Some preparations were made later from non-pyrogenic pigskin gelatin supplied by Dr. David Klein of the Wilson Laboratories.[2] The methods of preparation and properties of Oxypolygelatin given here apply to these two gelatins, unless otherwise specified.

The essential steps in the preparation of Oxypolygelatin are (1) decalcification of gelatin, if it contains more than about 0.02% calcium; (2) condensation with glyoxal; and (3) oxidation with hydrogen peroxide and sterilization by autoclaving. The detailed procedures for these steps are as follows:

a) Decalcification of Gelatin. — Gelatins from various sources contain different amounts of calcium. Removal of the calcium increases the reproducibility of preparations and may also eliminate some possible undesirable physiological effects.

[2]This gelatin was manufactured by the United Chemical and Organic Products Company, a division of Wilson and Company, Inc. It was designated as U-cop-co pyrogen-free gelatin.

Zeo-Karb Na, an ion-exchange material, was used for this purpose. It was placed in glass percolators and covered with saturated sodium chloride solution. After several hours it was washed with warm distilled water (60°–70° C.) until the washings were free of chloride ion, and then warm gelatin solution was allowed to filter through. It was found that on a laboratory scale 4 pounds of Zeo-Karb Na in an 8-inch percolator could remove the calcium from 30 liters of 7.0% gelatin solution, when the gelatin contained approximately 0.4% calcium.

b) Condensation.—A study was made of the reactions of gelatin with a number of bifunctional and polyfunctional coupling reagents, including phosgene, carbon suboxide, phosphoryl chloride, sulfur pentoxydichloride, and glucose and other polyhydroxy compounds, but all were discarded in favor of glyoxal, which is a simple, inexpensive reagent giving no by-products (except perhaps non-toxic traces of oxalate) which might be incompatible with transfusion. The condensation reaction between glyoxal and gelatin takes place over a wide range of *p*H and temperature. The optimum conditions and the reasons for their selection are described below.

Concentration of Gelatin.—Fve-percent gelatin solution was selected as the starting moterial, this being the concentration of Oxypolygelatin to be used for transfusion. Higher concentrations could have been used, but the increase in viscosity and tendency to gel would have been troublesome.

Concentration of Glyoxal.—The concentration of glyoxal chosen was the maximum amount which would react with the gelatin, under specified conditions, without producing a gel at any stage in the process of manufacture of the product. This value depends on the conditions of manufacture and also on the nature of the gelatin. The range of amounts of glyoxal for various gelatins was 0.005 to 0.05 grams of glyoxal per gram of gelatin.

Concentration of Hydrogen Ion.—The hydrogen-ion concentration was maintained between *p*H 7.0 and 4.5. At higher *p*H values the condensation reaction was slower than the reaction of protein hydrolysis. The condensation reaction was

accompanied by a fall in *p*H; hence the reaction mixture was adjusted to *p*H 7.0 after the addition of glyoxal and the *p*H was permitted to fall during the reaction.

Temperature.—Temperatures from 25° to 120° C. were tested. It was found that in the lower temperature range the reaction was too slow to be practical. Preparations made in the autoclave at 120° C. were satisfactory, but for convenient control and manipulation of large batches a vat method was used, and the reaction carried out at atmospheric pressure and at a temperature of about 95° C.

Time.—The time allotted for the reaction was critical only because of its effect on hydrolysis of the gelatin. Under the above conditions the average molecular weight, as determined by measurement of the osmotic pressure and the viscosity, reached a maximum at approximately 50 minutes. Continued heating, particularly when the *p*H had fallen to 5.0 or below, resulted in considerable reduction in the average molecular size of the final product.

c) Oxidation with Hydrogen Peroxide.—The oxidation of glyoxal-treated gelatin was accomplished in the first preparations by use of potassium permanganate and in all later preparations with hydrogen peroxide. The latter substance was preferred because no undesirable by-product was formed. The oxidation step, to be satisfactory, required the experienced control of peroxide concentration, hydrogen-ion concentration, time, and temperature.

Concentration of Hydrogen Peroxide.—The concentration of hydrogen peroxide was limited by several factors. Since hydrogen peroxide was found to destroy pyrogens (Campbell and Cherkin (1)) it was desirable to use at least 0.01 M H_2O_2, which was the minimal destructive concentration. The degree of oxidation had to be great enough to insure that the final preparation of OPG would remain liquid at temperatures as low as 15° C., but not so great as to produce excessive breakdown of the gelatin or to leave any free hydrogen peroxide after bottling and autoclaving. The practical range of concentration for the conditions adopted was found to lie between 0.01 and 0.02 g. of hydrogen peroxide per gram of gelatin.

Concentration of Hydrogen Ion.—A marked increase in acidity accompanied the oxidation reaction as well as the condensation, and it was hence necessary to adjust the solution to about *p*H 7.5 before adding hydrogen peroxide in order to avoid excessive degradation by acid hydrolysis. Under the above conditions the *p*H fell to about 5.5 in the final product. It was found that when a *p*H of 7.0 to 8.0 was maintained by the use of phosphate buffer the oxidation was too great, and the product was too highly degraded.

Time and Temperature.—The time allowed for the oxidation depended to some extent upon the amount of hydrogen peroxide solution used and upon the temperature. At temperatures below 60° C. many hours were required for the completion of the oxidation of polygelatin to a state in which the preparation was liquid at room temperature. For practical reasons the oxidation was carried out during the bottling and sterilization in the autoclave, which required 20 to 25 minutes at 120° C.

The foregoing information was obtained from experiments described in Tables 1 and 2.

TABLE 1

Properties of Oxypolygelatin (Series 130) Prepared from Knox C–1 Gelatin

Sample number	Grams reagent per gram gelatin		Relative viscosity*	Osmotic pressure*	Consistency of product		*p*H of product
	Glyoxal	Hydrogen peroxide			at 18°	at 5°C.	
130–AI	.002	0.0034	1.36	74	Liquid	Gel	6.87
AII	.002	.0170	1.22	106	Liquid	Gel	5.80
AIII	.002	.0340	1.17	65	Liquid	Gel	5.35
AIV	.002	.0680	1.13	100	Liquid	Liquid	5.04
BI	.005	.0034	1.51	69	Liquid	Gel	6.22
BII	.005	.0170	1.30	110	Liquid	Gel	5.65
BIII	.005	.0340	1.32	122	Liquid	Gel	5.42
BIV	.005	.0680	1.17	177	Liquid	Gel	5.16
CI	.010	.0034	1.93	39	Gel	Gel	6.00
CII	.010	.0170	1.53	75	Liquid	Gel	5.52
CIII	.010	.0340	1.31	86	Liquid	Gel	5.32
CIV	.010	.0680	1.22	116	Liquid	Gel	5.15
DI	.015	.0034	2.85	54	Gel	Gel	5.77
DII	.015	.0170	2.38	55	Gel	Gel	5.36
DIII	.015	.0340	1.36	91	Liquid	Gel	5.18
DIV	.015	.0680	1.19	142	Liquid	Gel	5.02

*1.0% solutions in 0.15 M phosphate buffer, *p*H 7.3, at 37°C.

Table 1 shows the effect of varying the concentrations of glyoxal and hydrogen peroxide used with a 5.0% solution of Knox (C–1) ossein gelatin. The gelatin was dissolved in warm water to concentration of approximately 7.0% and was then filtered through a Zeo-Karb Na column. The gelatin concentration was then accurately adjusted to 5.0%, as checked by the density of the solution.[3] The solution was then adjusted to *p*H 7.0 with 5 N NaOH and was divided into aliquots, and the flasks were placed in a boiling water bath. Different amounts of glyoxal, ranging from 0.002 g. to 0.015 g. of glyoxal per gram of gelatin, were added to four series of samples. The reaction was allowed to continue for 50 minutes, the samples were again adjusted to *p*H 7.0, and enough sodium chloride was added to give a final salt concentration of 0.9%. Different amounts of hydrogen peroxide were then added to samples of each of the four series containing varying concentrations of glyoxal, and the solutions were bottled and autoclaved for 20 minutes at 15 p.s.i. pressure.

Table 2 presents data of the same type for Wilson's gelatin. This gelatin contained only about 0.02% calcium, and the decalcification step was omitted. The results are similar to those obtained with Knox gelatin.

The data in the tables show the antagonistic action of glyoxal and hydrogen peroxide with respect to some properties. When a given polygel is treated with increasing amounts of peroxide the osmotic pressure increases and the relative viscosity, gelation, and retention (see physiology section) decrease. When different polygelatins are treated with the same quantity of peroxide the relative viscosity and gelation increase with increasing amounts of glyoxal; there is no pronounced effect on osmotic pressure.

d) A Typical Pilot-Plant Preparation (12676AY).—Preparation 12676AY, a pilot-plant preparation, gave about four

[3]Because of the variation in water content of gelatin an analytical method must be used to determine the protein concentration. The use of a pycnometer to determine solution density (Fig. 1) is as accurate as determining protein concentration by micro-Kjeldahl methods and is much less laborious.

hundred 500–ml. bottles of the product. Approximately 16.00 kg. of Wilson gelatin (non-pyrogenic, U-cop-co Lot 50980) was dissolved in 243 liters of distilled water contained in a 100-gallon glass-lined tank and kept in constant motion by a glass-coated agitator. The temperature was maintained at 90° C., and solution of the gelatin was completed in one hour. The volume of the solution was made up to 312 liters (taking into account a 4% reduction in volume at 25° C.) to give a 5.0% gelatin concentration, as determined by density measurement, and the *p*H was raised from 4.5 to 6.82 by the addition of 840 ml. of 5 *N* sodium hydroxide. Thirty minutes later 489 ml. of 30.6% (W/V) glyoxal was added, and also 384 ml. of 1.0 *N* sodium hydroxide. This amount of glyoxal was equivalent to 0.01 g. per gram of gelatin. The additional sodium hydroxide was required to neutralize the acid in the commercial glyoxal solution.

TABLE 2

Properties of Oxypolygelatins (Series 142) Prepared from Wilson Gelatin

Sample number	Grams reagent per gram gelatin		Relative viscosity*	Osmotic pressure*	Consistency of product		*p*H of product
	Glyoxal	Hydrogen peroxide			at room temperature	at 5°C.	
142–AI	0.0	0.0	1.33	62	Liquid	Gel	7.15
AII	.0	.0033	1.31	79	Liquid	Gel	7.00
AIII	.0	.0166	1.25	89	Liquid	Gel	6.20
AIV	.0	.0333	1.20	142	Liquid	Gel	5.65
AV	.0	.0666	1.15	165	Liquid	Liquid	5.12
BI	.005	none	1.35	68	Liquid	Gel	6.29
BII	.005	.0033	1.36	65	Liquid	Gel	6.10
BIII	.005	.0166	1.25	101	Liquid	Gel	5.53
BIV	.005	.0333	1.20	145	Liquid	Gel	5.23
BV	.005	.0666	1.14	195	Liquid	Liquid	4.93
CI	.01	none	1.39	57	Liquid	Gel	5.80
CII	.01	.0033	1.41	52	Liquid	Gel	5.66
CIII	.01	.0166	1.28	92	Liquid	Gel	5.20
CIV	.01	.0333	1.19	136	Liquid	Gel	5.00
CV	.01	.0666	1.15	190	Liquid	Liquid	4.80
DI	.02	none	1.58	49	Gel	Gel	5.45
DII	.02	.0033	1.97	45	Gel	Gel	5.44
DIII	.02	.0166	1.47	67	Liquid	Gel	5.02
DIV	.02	.0333	1.24	99	Liquid	Gel	4.81
DV	.02	.0666	1.15	180	Liquid	Liquid	4.63
Untreated	.0	.0	1.50	49	Gel	Gel	4.45

*1.0% solutions in 0.15 M phosphate buffer, *p*H 7.3, at 37°C.

Fifty minutes after the addition of glyoxal 2700 g. of sodium chloride, 504 ml. of 30% hydrogen peroxide (Special, Baker Lot 4645), and 300 ml. of 1.0 *N* sodium hydroxide were added. (The sodium chloride added was sufficient to give a 0.9% salt concentration, the hydrogen peroxide to give 0.01 g. per gram of gelatin, and the sodium hydroxide to neutralize the mixture at the start of the oxidation reaction.) Heating was then stopped and the mixture was allowed to cool to 70° C., which took 2.5 hours. It was then passed through filters, bottled, and autoclaved for 20 minutes at 120° C.

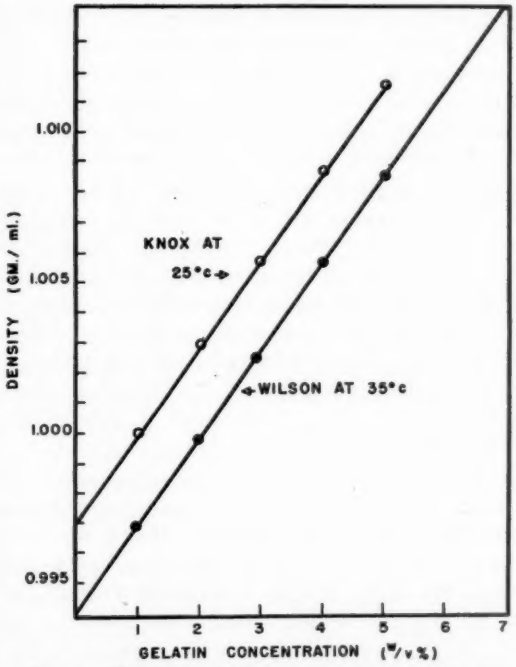

FIG. 1. The determination of protein concentration from pycnometer measurements.

A total of ten pilot-plant preparations were made. The osmotic pressure and viscosity values for these preparations are included along with retention values in Table 6 of Part II.

3. *Chemical and Physical Properties of Oxypolygelatin*

In order to obtain information concerning the nature of the changes involved in the conversion of gelatin to Oxypolygelatin, studies were made of some physical and chemical properties of the original gelatins and of the Oxypolygelatins. The results of these investigations are discussed below.

a) Isoelectric Point.—Isoelectric points may be measured in several ways, of which the observation of the moving boundary during electrophoresis is the least ambiguous. However, convection is likely to make such measurements difficult in the temperature range where gelatins and Oxypolygelatins are liquid, so that other methods were used.

These methods were (1) the viscosity-minimum method, in which the relative viscosity as a function of *p*H is measured and the point of minimum viscosity is taken as the isoelectric point; (2) the turbidity method (2), in which aqueous solutions of gelatin preparations at various *p*H are allowed to stand at about 2° C. and the *p*H for maximum turbidity, as judged by the eye in suitable lighting or measured with a turbidimeter, is taken as the isoelectric point; and (3) the microelectrophoresis method, in which the migration of ultramicroscopic particles in a protein solution at various *p*H values is observed in a Northrop-Kunitz cataphoresis apparatus, the point of zero mobility of the particles (presumably coated with protein) being determined by interpolation and taken as the isoelectric point.

Data obtained with the above methods, and given in Table 3, show that the isoelectric point of an Oxypolygelatin is lower than that of the parent gelatin. This is due in part to the fact that hydrolysis by autoclaving lowers the isoelectric point. Thus, for Knox gelatin autoclaved 4 hours at *p*H 7 and 120° C. and for Wilson gelatin autoclaved 3 hours at *p*H 7 and 120° C. the isoelectric points dropped 1.2 and 2.2 *p*H units, respectively. However, similar treatment of Oxypolygelatin 11382Y did not further lower the isoelectric point. Other effects which might lower the isoelectric point are the binding of amino and guanidino groups by glyoxal and the production of carboxyl groups during the peroxide oxidation.

TABLE 3

Isoelectric Points of Modified and Unmodified Gelatins

Preparation	Isoelectric point	Method of determination
Human serum albumin Harvard Run 404	4.6	Microelectrophoresis
Knox Gelatin, Lot C-1	4.5	Microelectrophoresis
Knox Gelatin, Lot C-1 (autoclaved)	3.3	Microelectrophoresis
Wilson Gelatin, 50980	7.8	Viscosity minimum
Wilson Gelatin, 64032	6.6	Microelectrophoresis
Wilson Gelatin, 64032 (autoclaved)	4.4	Microelectrophoresis
Oxypolygelatin, 11382Y	3.7	Microelectrophoresis
Oxypolygelatin, 11382Y	3.9	Viscosity minimum
Oxypolygelatin, 11382Y	4.0	Turbidity maximum
Oxypolygelatin, 11382Y (reautoclaved)	3.7	Microelectrophoresis
Oxypolygelatin, 12676AY	4.2	Microelectrophoresis

Further data on isoelectric points are discussed in the section on fractionation and studies on the heterogeneity of OPG.

b) Electrophoretic Behavior.—Although some study was made of the electrophoretic behavior of gelatins and Oxypolygelatins, the significance of the data is doubtful because of the necessity of partially hydrolyzing the preparations to give soluble products at 3° C. Schlieren patterns from the partially hydrolyzed preparations showed considerable homogeneity for the Knox and Wilson gelatins and for the Oxypolygelatins. The samples were run in phosphate buffer at *p*H 7.3 and ionic strength 0.1, the electrophoresis being carried out for 100 minutes. The OPG samples showed much higher mobility than the parent materials, but essentially the same degree of homogeneity.

c) Viscosity.—One of the primary goals in the investigation was to obtain a product liquid at room temperature. There is a qualitative correlation between the viscosity of a gelatin solution and its tendency to gel; in general, solutions of high viscosity tend to gel at higher temperatures than those of low viscosity. In the evaluation of an Oxypolygelatin the consistency (liquid or gel) of a 5% solution at 20° C. was

determined visually and the relative viscosity in acetate buffer of ionic strength 0.10 at *p*H 5.0, 37° C., and 1% protein was measured in a capillary viscometer.

The effects of autoclaving, condensation with glyoxal, and oxidation by hydrogen peroxide can be seen in the data of Tables 1 and 2. Autoclaved gelatin (AI) was a liquid at room temperature and had a much lower viscosity than untreated gelatin. Reaction with glyoxal raised the viscosity while oxidation with hydrogen peroxide reduced it. The effect of hydrogen peroxide on viscosity is shown in Fig. 2. Two features of this graph are especially interesting. First, it may be seen that low concentrations of hydrogen peroxide seem to favor the condensation reactions, and second, that with higher concentrations of hydrogen peroxide a constant limiting viscosity is approached, which is independent of the glyoxal concentration.

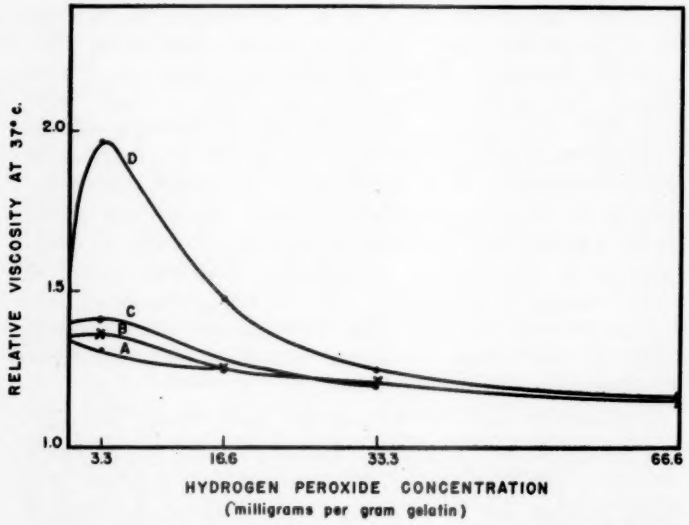

FIG. 2. The effect of hydrogen peroxide concentration on the viscosity of gelatin and polygelatin solutions. The curves A, B, C, and D are obtained from the data of Table 2 in which the gelatin and polygelatin solutions are represented as series A, B, C, and D.

d) Osmotic Pressure.—The osmotic pressure is greatly changed by conversion of gelatin to Oxypolygelatin. The condensation with glyoxal increases the average molecular weight and decreases the osmotic pressure, and opposite effects are produced by oxidation with hydrogen peroxide. The measurements tabulated in Table 1 were made with open osmometers and cellophane membranes. The equilibrium height of the liquid column was measured directly and the osmotic pressure was calculated after correcting for capillarity. Determinations were carried out at *p*H 7.4 in phosphate buffer of ionic strength 0.10 and 37° C. The Oxypolygelatins were isoelectric in the region of *p*H 4.0 to 4.5. The magnitude of the Donnan pressure was determined and found to be small.

The osmotic pressure values in Table 2 were obtained by the use of an osmometer patterned after that of Sørensen (3) and illustrated in Fig. 3. The solution is introduced into the cellophane bag A, ¾ inch in diameter and 2 inches in length, which is secured to B, a glass holder connected to the manometer H. Beaker C is placed in a constant-temperature bath (± 0.02° C.). Bottle J is a constant-level device which replaces water into C as it evaporates.

After filling the bag A and osomometer B so that the liquid level stands above stopcock F, the system is allowed to approach equilibrium for about 12 hours with F closed. After this time the liquid level at E is observed through a cathetometer and stopcock F is cautiously opened while adjusting the position of I to keep the level at E constant. By measuring the difference in level between E and the solvent in C, and subtracting the capillary rise, the difference in pressure in the two sides of the membrane A is found. This pressure is observed at intervals and plotted as a function of time and the equilibrium pressure is then found by extrapolation.

When water was used in the manometer a small amount of Aerosol or some other detergent was added to insure uniformity of wetting of the capillary walls. (The capillary rise at E may be lowered to about 5 mm. of water by introducing a trace of Aerosol in the capillary tube.)

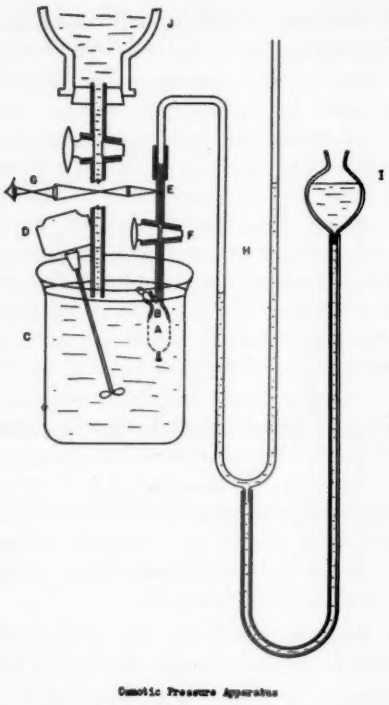

FIG. 3. The modified Sørensen osmometer used to obtain the osmotic pressure data reported in text.

To determine the accuracy of the method, human serum albumin (Harvard Run 404) was dissolved in acetate buffer at *p*H 5.0 and ionic strength 0.10 to give a 1% protein solution. The osmotic pressure was then measured against the same acetate buffer. The beginning of dialysis corresponds to zero time in Fig. 4. After the measurement the solution was analyzed for protein, using a nitrogen factor of 6.25. The measurement was carried out at 37° C. Each line in the graph corresponds to a separate osmometer. The data yielded a value of 71,000 for the molecular weight of human serum albumin (accepted value = 69,000). The over-all experimental error was found to be about 5%.

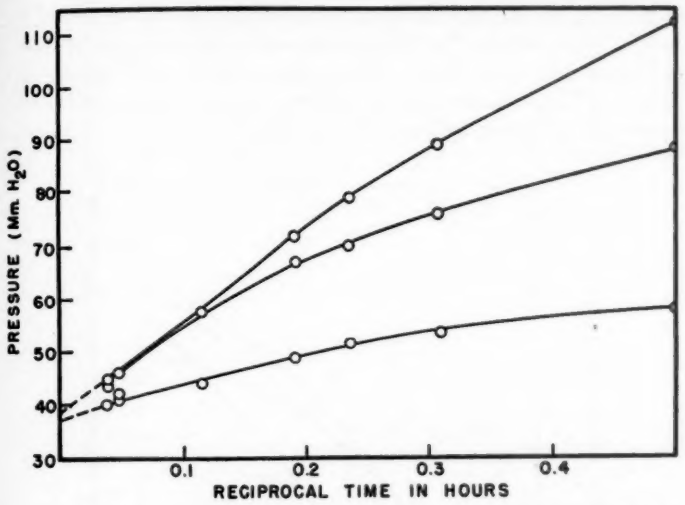

FIG. 4. Osmotic pressure of 1.0% solutions of human serum albumin in acetate buffer at *p*H 5.0, ionic strength of 0.10 and 37° C. The different curves merely represent 3 different osmometers.

It must be emphasized that inasmuch as the Oxypolygelatins as gelatins are polydisperse and the membranes used were permeable to molecules of small size, the measurements obtained apply only to those molecules which could not pass through the bag readily. The fraction which passed readily varied from about 1% to 20% of the total (as determined by nitrogen analyses) in the time required to attain equilibrium pressure. The changes in osmotic pressure produced by condensation and oxidation of glyoxal-treated gelatins (Table 2) are shown in Fig. 5, which is a plot of data from measurements made near the isoelectric points of 1% protein in buffers of ionic strength 0.01.

The contribution of the Donnan pressure for Oxypolygelatins and for untreated Wilson's gelatin was small in the buffer used, even far from their respective isoelectric points. Thus, the osmotic pressure of OPG 12676AY was 87 mm. of water (1% protein) at its isoelectric point (*p*H 5) and 90 mm. at *p*H 7.4. For untreated Wilson gelatin, with isoelectric point

at *p*H 7.8, the osmotic pressure was 49 mm. of water at *p*H 5 and 52 mm. at *p*H 7.4. This difference is practically within experimental error. The slope of curves of the P/C against C shown in Fig. 6 is considerably greater than is usually obtained for serum albumin, hemoglobin, or casein.

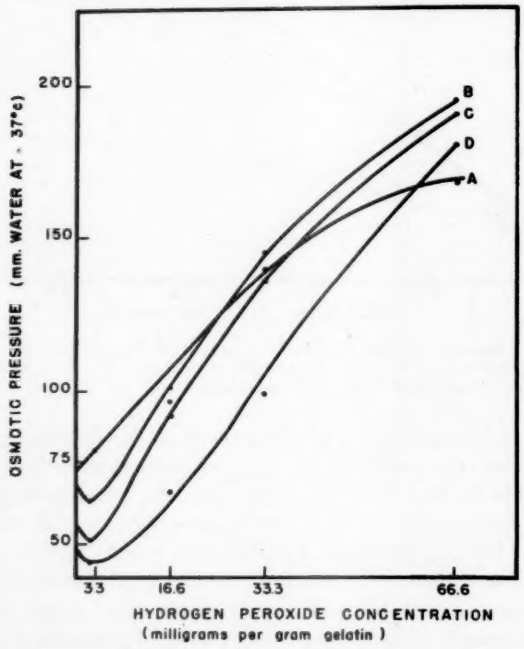

FIG. 5. The effect of hydrogen peroxide concentration on the osmotic pressure of gelatin and polygelatin solutions from data in Table 2. Curves A, B, C, and D represent series of the same letters in the table.

e) Molecular Size and Shape.—Calculations of molecular size and shape from osmotic pressure and viscosity data are reliable only for monodisperse systems or for polydisperse systems in which the particle size distribution is known. Although these factors have not been established for OPG some qualitative statements can be made from the data of Table 2

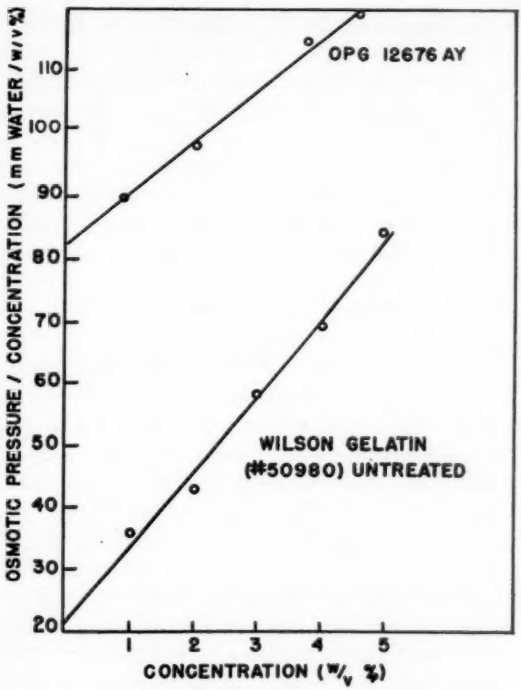

FIG. 6. The relationship of protein concentration to osmotic pressure of OPG 12676AY and its parent gelatin 50980.

From the relative viscosities for 1% protein concentration an approximate intrinsic viscosity (2) defined by Kraemer and Lansing (4) as $[\eta] = \lim_{C \to 0} (\frac{\eta_r - 1}{C})$ may be calculated by dividing the relative viscosity with respect to water by 1.06 and subtracting 1.00. The factor 1.06 is the viscosity of the acetate buffer relative to water. From a comparison of gelatins or Oxypolygelatins of equal viscosity or of equal osmotic pressure, such as untreated gelatin ($[\eta]=0.14$) and DI ($[\eta]=0.49$), both of which had an osmotic pressure of 49 mm., it appears that the molecular length of a relatively small fraction consisting of very long molecules is being made still greater.

This is also borne out by a comparison of BI and DIII (Table 2).

The effect of small amounts of hydrogen peroxide can be appreciated by comparing Polygel C$[\eta]$=0.54 and osmotic pressure 41.0 mm., with OPG DII, $[\eta]$=0.86 and osmotic pressure 45 mm. This would indicate that long molecules tend to become even longer and that only a small amount of low molecular weight material is formed, since the osmotic pressure rises by only a small amount after conversion of gelatin to OPG.

The change of osmotic pressure with concentration provides an index of the non-ideality of a solute. For an ideal solute the slope of the line which results from plotting the ratio of osmotic pressure to concentration versus the concentration is zero. For a non-ideal solute this line has a positive slope, which is large for long chain polymers, and is small for such solutes as serum albumin. From the graphs of P/C versus C for untreated Wilson gelatin and Oxypolygelatin 12676AY (Fig. 6) it may be seen that the slope of the curve is somewhat greater for Wilson gelatin than for the corresponding Oxypolygelatin, suggesting that the molecules of the latter have a smaller axial ratio than those of untreated gelatin. (For further discussion of theoretical aspects see 4a,b,c.)

f) Ethanol Fractionation.—It was apparent from some preliminary studies made by Professor J. W. Williams on Oxypolygelatins that treatment with glyoxal and hydrogen peroxide increases the heterogeneity of the gelatin with respect to molecular size distribution. The following experiments were then made to obtain some idea of the distribution of these various components as determined by alcohol precipitation. An ethanol fractionation of Oxypolygelatin 11382Y was carried out. Analysis of the parent material gave the result:

Total solids (dried to constant weight over P_2O_5)	5.68% (W/V)
Total ash (presumably NaCl)	0.90%
Total nitrogen (Nesslerization)	0.807% (W/V)
Nitrogen factor	5.96%
Protein concentration	4.82%
Density at 21° C.	1.007 g./ml.

A preliminary fractionation was carried out to determine the relation between volume of ethanol added and quantity of protein precipitated. Ethanol (95%) was added slowly to 400 ml. of Oxypolygelatin 11382Y, initially at *p*H 4.00 and ionic strength 0.15. The temperature was maintained at 22° C. The time allowed for equilibrium to be attained varied from 0.5 to approximately 12 hours. When a precipitate was separated after 0.5 hours no additional precipitate appeared in the supernatant after standing overnight. The resulting precipitates (viscous gels) were centrifugal off and analyzed for total nitrogen. After 1500 ml. ethanol had been added and the precipitate had been removed, the supernatant was cooled to 4° C., and additional precipitate formed. About 80% of the original protein had been precipitated up to this point and 17% was found in the final supernatant, giving a total recovery of 97%.

A larger separation was undertaken to provide an amount of each fraction sufficient for characterization. The starting material was 2.00 liters of Oxypolygelatin 11382Y at *p*H 4.00 and ionic strength 0.15. The temperature was maintained at 20° C. ± 1.0 throughout the room-temperature part of the fractionation. Fractions which precipitated as a syrupy liquid were centrifuged off, reprecipitated from ethanol, and dried to constant weight *in vacuo*. Details of the fractions are given in Table 4.

TABLE 4

Physical and Chemical Properties of Ethanol Fractions of Oxypolygelatin 11382Y

Fraction	Per cent ethanol	Approximate per cent of total protein	Isoelectric point[4]	pH when dissolved in water	Consistency of 5% soln. at 1°C.[5]	Osmotic pressure 1% protein mm H_2O	Number-average molecular weight (M_n)
I	58	8.2	3.90	4.30	Firm gel	67	39,000
II	65	11.5	3.95	4.25	Firm gel	64	41,000
III	68	11.5	3.85	4.30	Firm gel	61	43,000
IV	70	13.7	3.85	4.33	Firm gel	83	32,000
V	73	10.7	3.90	4.35	Firm gel	90	29,000
VI	76	11.0	3.90	4.32	Soft gel	128	21,000
VII	80	7.0	3.80	4.32	Syrup	162	16,000
VIII[1]	..	10.4	3.90	4.32	Liquid	208	13,000
IX[2]	..	15.0	3.55	4.63	Liquid	250	10,000
X[3]	..	1.0	3.35	4.63	Liquid	...	.....

[1]Precipitate formed from VII supernatant upon cooling to 4°C.
[2]Supernatant from VII evaporated in vacuo and extracted with 95% ethanol, giving insoluble IX and soluble X.
[3]Based on total protein recovered.
[4]Determined by microelectrophoresis.
[5]All fractions were liquid at room temperature.

The recovery of total solids was approximately 90%, as was expected from preliminary analyses of the small sample. Fractions I to IX were fluffy or granular solids, readily soluble in water at room temperature, and fraction X was a sticky brown residue. Nitrogen factors varied around a value of 5.9 based on the nitrogen content of weighed samples.

The osmotic pressure of the ethanol fractions of Oxypolygelatin 11382Y was determined in the apparatus already described, using approximately 1.0% protein. The solvent was acetate buffer, *p*H 4.00, ionic strength 0.10, at 37° C.; 0.01% Merthiolate (Lilly) was present in all solutions. Fractions I to VIII reached equilibrium in less than 24 hours and the osmotic pressure remained constant within 2 mm. H_2O for an additional 24 hours. The osmotic pressure of IX continued to drop steadily even after 93 hours, the protein concentration falling from 1.2% to 0.52%. The calculated average molecular weights of the gelatin fractions show an antibatic relation to solubility in alcohol.

Solution

Viscosities were measured as a function of concentration in a 10-ml. Ostwald viscometer by the method previously described. All solutions were in acetate buffer, *p*H 4.00 and ionic strength 0.10, at 37.6° C.; 0.01% Merthiolate was present. Table 5 gives viscosities and derived quantities for the fractions. On the assumption that the fractions are nearly homogeneous, fractions VII, VIII, and IX appear to follow Staudinger's law rather well, but these fractions cover only a narrow range of molecular weights. However, the significantly larger value of the ratio $M_n/[\eta]$ for the higher molecular weight fractions may be interpreted to mean that $[\eta]$ is not so large as would be expected for a given number-average molecular weight. Such a conclusion may be explained by the assumption that the larger molecules are more nearly spherical than the smaller ones. (Note the high value of $M_n/[\eta]$ for human serum albumin.)

Inasmuch as the osmotic pressure data for the Oxypolygelatin fractions are known at only one concentration (1%),

the values of M_n given in Table 5 are only rough estimates of the molecular weights. For Oxypolygelatin 12676AY the molecular weight estimated from the osmotic pressure of a 1% solution would be 30,000 instead of the value of 32,000 obtained from the ratio of P/C at zero concentration from Fig. 6.

TABLE 5

Viscosity and Related Properties of Ethanol Fractions of Oxypolygelatin 11382Y

Fraction	Protein conc., C, %	Relative viscosity η_r	$\frac{\eta_r - 1}{C}$	Intrinsic viscosity $[\eta]$	$\frac{M_n}{[\eta]} \times 10^{-2}$*
I	0.890	1.268	0.301		
	0.445	1.123	0.277	0.250[1]	156
	0.223	1.059	0.264		
II	0.920	1.252	0.274		
	0.460	1.117	0.255	0.248[1]	165
	0.230	1.058	0.252		
III	0.896	1.214	0.239		
	0.448	1.104	0.232	0.215[1]	200
	0.224	1.050	0.223		
IV	0.938	1.188	0.200		
	0.469	1.090	0.192	0.190[1]	169
	0.235	1.045	0.191		
V	0.915	1.162	0.177		
	0.458	1.078	0.170	0.170[2]	170
	0.229	1.039	0.170		
VI	0.915	1.138	0.151		
	0.458	1.066	0.144	0.146[2]	144
	0.229	1.039	0.148		
VII	0.909	1.113	0.124		
	0.455	1.056	0.123	0.123[2]	130
	0.237	1.028	0.123		
VIII	0.948	1.100	0.105		
	0.474	1.050	0.105	0.105[2]	124
	0.237	1.025	0.105		
IX	1.22	1.093	0.076		
	0.610	1.047	0.077	0.077[2]	130
	0.305	1.023	0.077		
OPG 11382Y	0.960	1.160	0.167		
	0.480	1.078	0.162	0.162[2]	129
	0.240	1.039	0.162		
Human serum albumin†	....	....	....	0.049	1390

*See Table IV for values of M_n.

†From Cohn, E. J., and Edsall, J. T. "Proteins, Amino Acids and Peptides."

[1]Evaluated graphically.

[2]Evaluated numerically.

However, the data on viscosity and osmotic pressure for Fractions III to VIII inclusive give a nearly straight line when log M is plotted against log $[\eta]$ (Fig. 7). The relationship can be expressed in the form of the equation

$$[\eta] = KM^a \text{ with } K = 3.02 \times 10^{-4} \text{ and } a = 0.62.$$

This value of a is in the same range as that found for other high polymers(5).

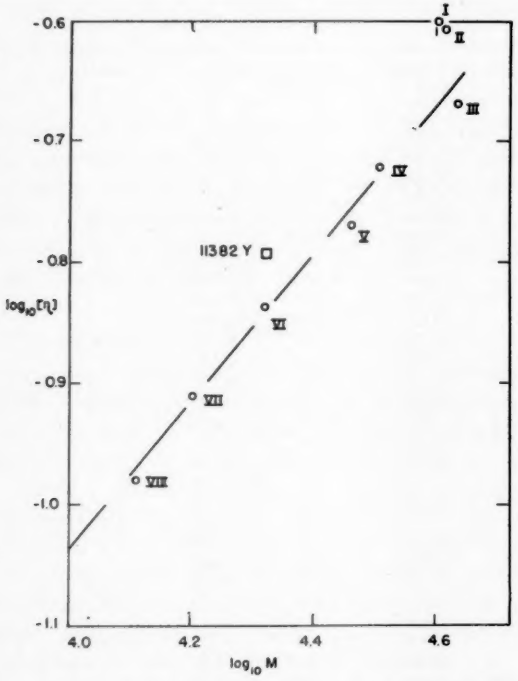

FIG. 7. The relationship between molecular weight (by osmotic pressure) and viscosity of ethanol precipitated fractions of OPG 11382Y.

In order to ascertain whether the ethanol fractionation of Oxypolygelatin 11382Y had produced drastic changes in its physical properties a number-average molecular weight was calculated from the molecular weights of the fractions and the

weights of each fraction found. The following equation gives the number-average molecular weight M_n in terms of M_i, the molecular weight of fraction *i*, and W_i, the weight of fraction *i*:

$$M_n = \frac{\Sigma_i W_i}{\Sigma_i \frac{W_i}{M_i}}$$

The calculated value of M_n is found to be 20,000, whereas the value for intact Oxypolygelatin 11382Y estimated from osmotic pressure at 1% protein is 21,000.

Fraction X was excluded from the average calculated above since this fraction is probably completely dialyzable and would not affect the direct determination of the average molecular weight of Oxypolygelatin 11382Y by osmotic pressure measurement. This supposition is supported by the observation that much of the material of fraction IX is also dialyzable.

It was concluded that the fractions were substantially unmodified by the separation procedure.

g) Digestion by Enzymes.—In order to determine whether there are differences in digestibility between untreated gelatin and Oxypolygelatin, experiments were carried out on the hydrolysis of gelatins in the presence of pepsin and trypsin. The enzymes used were crystalline pepsin and trypsin diluted with magnesium sulfate (Lehn and Fink Company). The enzyme solutions were made up in 0.15 *M* NaCl (10 mg. of enzyme mixture per ml. of solution) at *p*H 6 for trypsin and at *p*H 3 for pepsin. The enzyme concentrations studied ranged from 2 to 7 ml. of enzyme solution per 100 ml. of 5% gelatin or Oxypolygelatin. The digestions were carried out at 25° C. and *p*H 7.6 for trypsin and at 25° C. and *p*H 2 for pepsin. Thymol was used as a preservative. At intervals 10-ml. samples of the digestion mixture were removed and brought to *p*H 7.0; 5 ml. of formaldehyde solution (20% W/V HCHO at *p*H 9.0) was then added to each sample, and the mixture was titrated to *p*H 9.0 with 0.10 *N* NaOH.

The results are plotted in Fig. 8 to show titration values as a function of time for a mixture of 40 ml. of 5% gelatin or Oxypolygelatin and 1 ml. of trypsin solution. The numbers AI and CIII refer to preparations in Table 2, AI being autoclaved gelatin and CIII an Oxypolygelatin. The data show that the number of free amino groups in CIII is slightly less than that in AI and that CIII is digested more slowly than AI. When the digestion was carried out for about 48 hours, using 100 ml. of protein solution (5%) and 2 ml. of trypsin or pepsin solution initially, with 5 ml. more enzyme added after 24 hours, the following final titration values (ml. of 0.10 *N* NaOH) were obtained:

AI—trypsin	2.38 ml.
CIII—trypsin	1.57 ml.
AI—pepsin	0.35 ml.
CIII—pepsin	0.23 ml.

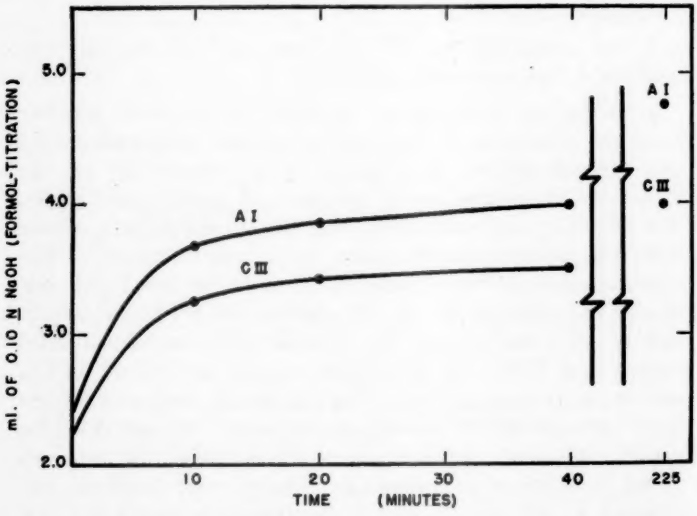

FIG. 8. The rate of digestion of gelatin AI and OPG CIII (Table 2) by trypsin as indicated by formol titration.

These data confirm the previous results and show that the particular Oxypolygelatin studied was apparently about 66% as digestible by crystalline pepsin or trypsin as autoclaved gelatin. However, when comparison is based on the fraction of the total reaction completed in a given time there was little difference between the two preparations. These results indicate that the chemical treatment has changed about 34% of the enzymatically hydrolyzable peptide groups of gelatin.

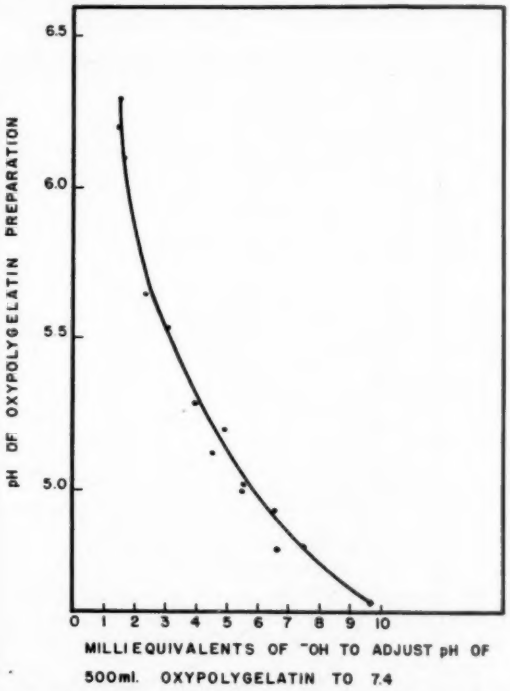

FIG. 9. Amount of hydroxide ion required to adjust the various OPG preparations in Table 2 to *p*H 7.4.

h) Neutralization Equivalents.—Accompanying the drop in isoelectric point there was a large drop in *p*H during both con-

densation and oxidation. Thus, if condensation was begun with a gelatin solution at *p*H 7.4 and neutral glyoxal was used the *p*H of the polygel would be in the region 5 to 6, and after oxidation it would have dropped several tenths of a *p*H unit more.

The low *p*H of the final product does not seem to be a serious objection to its use as a blood substitute: the amount of hydroxide ion needed to bring the *p*H of 500 ml. of Oxypolygelatin, Lot 12676AY, to 7.4 is only 3.92 milliequivalents, and normal human serum contains about 27 milliequivalents of

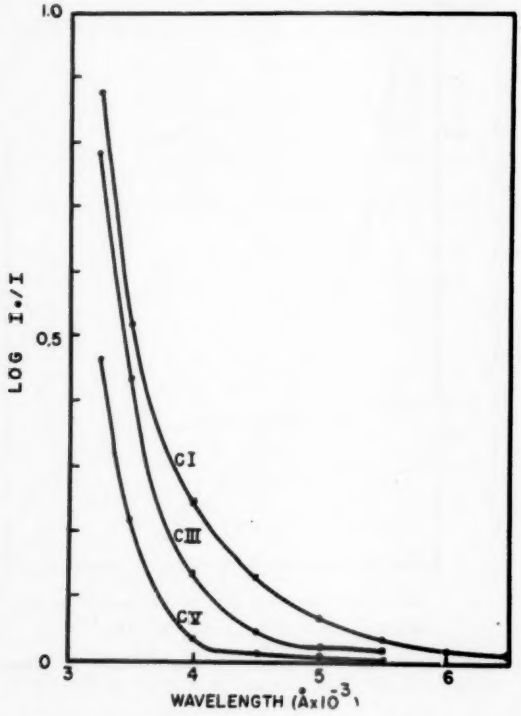

FIG. 10. Light absorption as a function of wave length of polygelatin CI and Oxypolygelatins CIII and CV Table 2 as measured with a Beckman spectrophotometer.

bicarbonate ion per liter. The final *p*H of the Oxypolygelatin and the alkali equivalent (milliequivalents of hydroxide ion needed to adjust 500 ml. of 5% Oxypolygelatin to *p*H 7.4) are related as shown in Fig. 9, representing data from Table 2.

i) Light Absorption.—The effects of the reaction between gelatin and glyoxal and of the oxidation of the condensation product are evidenced by changes in color. During the glyoxal treatment the color deepens to brown, or, if large amounts are used (0.04 g. of glyoxal per g. of gelatin), to nearly black, indicating that conjugated unsaturated systems are being formed. However, the nature of this reaction is not known.

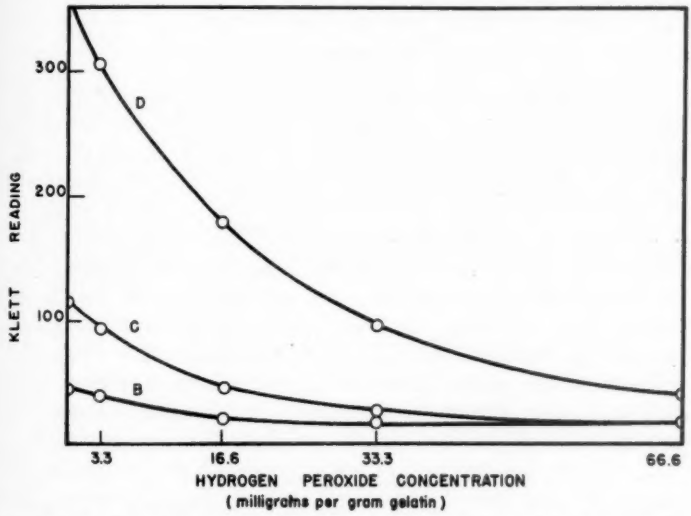

FIG. 11. Light absorption of polygelatins BI, CI, and DI and the effect of hydrogen peroxide in each series (Table 2). Values were measured with a Klett colorimeter using a 400–465 Mμ filter.

The subsequent oxidation with hydrogen peroxide tends to lighten the brown color, and if sufficiently large amounts are used the product is practically colorless. The plot of light absorption of Oxypolygelatins as a function of wave-length shown in Fig. 10 illustrates these facts. The numbers of the Oxypolygelatins refer to Table 2. The absorption of the poly-

gelatin CI is considerably higher than that of the OPG 142-CV. These measurements were made with a Beckman Quartz Spectrophotometer, using 1-cm. silica glass cells. Protein concentrations were 1% in acetate buffer at *p*H 5.0 and ionic strength 0.10.

Other measurements of light absorption were made using the Klett-Summerson Photoelectric Colorimeter. The blue filter No. 42, having a transmission range of 400–465 millimicrons, was employed. Protein concentration and solvent were the same as above. The data plotted in Fig. 11 show the effect of hydrogen peroxide on the light absorption of glyoxal-treated gelatins in Table 2.

4. *Summary*

The foregoing section describes the preparation of Oxypolygelatins which were subsequently used in the physiological and clinical tests described in Part II. The experimental basis for the selection of certain steps in manufacture of pilot-plant batches is discussed, as are some of the pertinent physical properties of the resulting products. Little is known as yet regarding the basic nature of the reactions which take place in the treatment of gelatin with glyoxal and an oxidizing agent. One can only speculate from indirect evidence given by osmotic pressure, viscosity, and retention studies (Part II) that the shape of the molecule has been changed from a long rod to a more branching structure. The essential changes in molecular configuration might be first, the formation of a large brush-like framework as a result of the condensation of many long gelatin molecules by glyoxal, and second, the fragmentation of this framework into molecules having about the same size as the original gelatin but of more symmetrical shape.

PART II. PHYSIOLOGICAL STUDIES

1. *Introduction*

In the previous section the details of manufacturing and some of the physical properties of Oxypolygelatin have been pre-

sented. The physiological studies which were made for controlling the various preparations and measuring their efficacy as a plasma substitute are given in this section.

As was pointed out previously, gelatin has most of the properties which would be required of a good plasma substitute but has the undesirable property of gelling at usual room temperatures. Gelling can be reduced by partial hydrolysis with heat, but the consequent reduction in molecular size then permits too rapid diffusion from the blood stream. Since the problem therefore was to reduce gelation without an attendant decrease in retention in the circulation the physiological studies were concerned chiefly with the rate of disappearance of various preparations of gelatins and modified gelatins from the blood stream. Aside from toxicity tests, which were made on each important preparation, no physiological test other than retention was routinely made. The few tests made for possible antigenicity as well as the blood pressure studies made with a few of the more important preparations will be described briefly. Finally, a limited number of clinical tests are described; these are essentially tests for tolerance in humans, since in most cases the patients were not in shock or in need of transfusion.

2. *Methods Used in Retention Tests*

In order to obtain comparative retention data for the respective preparations it was obvious that a standard procedure had to be developed for treatment of animals and for analysis for gelatin in the blood. Although some of the procedures were necessarily empirical, they were standardized as well as possible so that good comparative data were obtained.

a) Hemorrhage, Transfusion and Sampling.—The necessity for bleeding animals prior to the administration of the plasma substitute was indicated by the results of Price *et al.* (6) as well as our own experiments, in which it was found that gelatin and other substances are removed faster from the circulation of a normal animal than from one previously subjected to hemorrhage. Although dogs were used in preliminary

studies, subsequent testing was carried out entirely with rabbits. The rabbits were unselected as to sex or breed and ranged in weight from 2.5 to 4.0 kg.

An initial volume of blood amounting approximately to one-third (20 ml./kg.)[1] was withdrawn by cardiac puncture into a syringe containing about 3 ml. of 10% sodium citrate to prevent coagulation. The plasma was separated by centrifugation and preserved for future analytical operations. Immediately after the hemorrhage an equal volume of 5% belatin substitute was injected via the marginal ear vein, and the animal was placed in a cage which contained neither food nor water.

Blood samples for the estimation of plasma volume and gelatin concentration were obtained 5 and 24 hours after the transfusion. The animal was anesthetized with ether, and an incision was made in the region of Scarpa's triangle, the femoral artery and vein being exposed. Then 8 ml. of blood was drawn from the femoral artery into a calibrated 10-ml. syringe containing 2 ml. of a mixed oxalate anticoagulant (6). The contents were mixed and delivered into a 15-ml. graduated centrifuge tube. Five ml. of Evans' Blue (T-1824) solution, containing 0.2 mg. of dye per ml., was then injected into the femoral vein, and 15 minutes later another 8-ml. sample of arterial blood was taken in the usual manner. This sample was used for the determination of hematocrit and of the concentrations of dye and gelatin. At the end of the sampling operations the femoral artery was ligated distally to the profunda and the incision was sutured.

b) Determination of Cell, Plasma and Blood Volumes.—Hematocrit values (H) were determined directly in the 15-ml. centrifuge tubes by reading the volumes of packed cells after centrifuging the blood samples for 45 minutes at 2,000 r.p.m. Suitable corrections were made for the dilution of the plasma by the anticoagulant solution by means of the following expression:

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[1]This assumption does not entail too great an error if the animal's weight is between 2.5 and 4.0 kg.

$$H = \frac{\text{Vol. of cells}}{\text{Vol. of fluid} - \text{Vol. of anticoagulant}}$$

The need for a precise estimate of the plasma volume at the time of sampling follows from the unstable condition of the circulation following hemorrhage. Although it is possible to extrapolate values for plasma volume from a previous dye injection when the circulation is at equilibrium by assuming a constant rate of dye loss (6, 7), it is erroneous to make such estimates when the animal has been subjected to extensive hemorrhage and has been transfused with a foreign protein of considerable osmotic activity. Plasma volume was therefore estimated on aliquots of the supernatants obtained in the hematocrit determinations. Optical densities of pre- and post-dye samples at 620 mμ were determined with a Beckman photoelectric spectrophotometer. After subtracting blank readings and correcting for dilution with anti-coagulant, the post-dye values were referred to a corrected calibration curve from which plasma volume could be obtained directly. Hemolysis rarely occurred, but when present a correction could be applied from readings taken at 540 mμ. The blood volume (BV) was calculated from the plasma volume (PV) and hematocrit by the expression

$$BV = \frac{PV}{100 - \text{hematocrit}}$$

A calibration curve relating optical density to concentration of dye was established by measuring the optical densities of 5-ml. aliquots of two plasma samples and one sample of distilled water to which had been added various volumes (0.02–0.20 ml.) of T-1824 solution containing 0.2 mg. of dye per ml. After correction for the appropriate blanks the plot was linear with respect to the volume of dye added (Fig. 12). The dilution for each point was calculated and was multiplied by the ml. of dye used in the injection, and a curve was plotted relating plasma volume to optical density (Fig. 13). This curve was then corrected for dye disappearance occurring within 15 minutes by means of the average rate of dye loss

estimated from time-dilution studies made on three rabbits. The average rate of dye disappearance from the circulation, according to our studies, was 0.90 percent per minute.

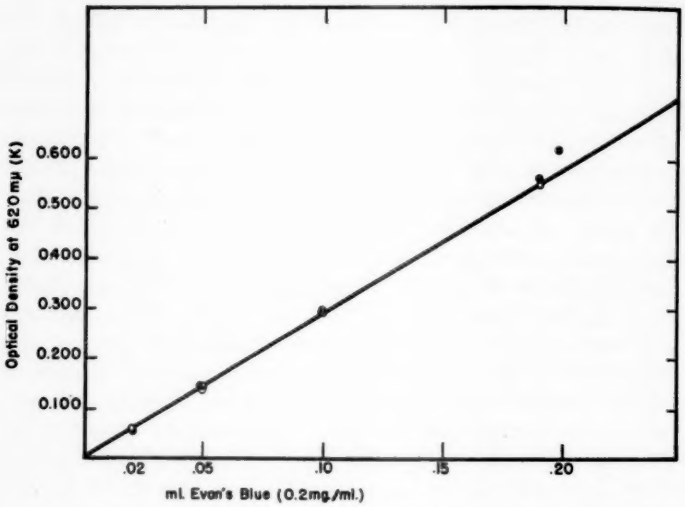

FIG. 12. Standaridization curve for Evans Blue (T-1824). When added to 5.0 ml. of rabbit plasma (o) or 5.0 ml. of water (•).

c) Gelatin Determination.—The concentration of gelatin in the oxalated post-dye plasma samples was determined by a colorimetric micromethod (8), based on the difference in the capacities of plasma proteins and gelatin to produce color with the Folin-Ciocalteu phenol reagent. The color developed is due to the action of the reagent on the aromatic amino acid residues which are abundantly present in the plasma proteins but are found only to a limited extent in gelatin.

The determination of gelatin in this investigation involved the separate analyses of a tungstic acid precipitate (representing total proteins) by two independent methods: (a) the Kjeldahl-Nessler, which depends on the nitrogen content of the precipitate and (b) the colorimetric method based on the

detection of aromatic residues by the Folin-Ciocalteu reagent. Because plasma proteins and gelatin are similar in their nitrogen content but different in their content of aromatic residues, it was possible to establish a curve relating the aromatic residue content of a mixed precipitate to the percentage of the total protein as gelatin.

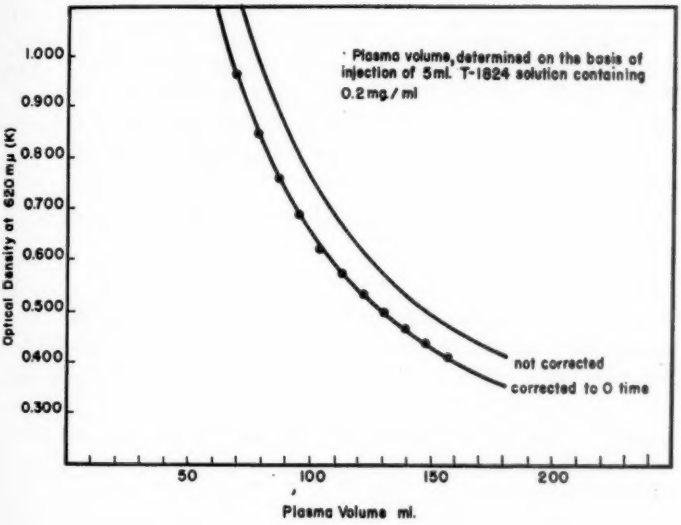

FIG. 13. Calibration curve for plasma volume estimated from optical density of dye-plasma mixtures.

Calibration experiments made on a series of mixtures, variable in their plasma protein/gelatin ratios but constant in respect to the concentration of total protein, revealed that a plot of specific Folin color (*i.e.*. the color developed per milligram of protein) against the protein complex expressed as percent gelatin is a straight line passing through the values for plasma protein and for gelatin. Such linear relationships, demonstrated in several calibration experiments, made it possible to refer the specific Folin color of a post-transfusion

plasma sample, presumed to contain gelatin, to a composition curve established from the analytically determined specific Folin color values of a pre-transfusion plasma sample and a sample of the gelatin administered in the particular experiment. The advantage of this method is that no fractionation of gelatin and plasma proteins is required, obviating errors due to coprecipitation characteristic of the differential precipitation with trichloroacetic acid as used by Taylor and Waters (9).

From knowledge of the plasma volume and the gelatin concentration the amount of gelatin remaining in circulation may be obtained. The retention of a gelatin is defined as the percent of injected gelatin present in the circulation at a given time. The retention values have an estimated probable error of 10%.

A typical protocol is presented to illustrate the method of calculating the retention.

Rabbit 400 weighing 3.0 kg. was bled of 57 ml. by cardiac puncture. Following the hemorrhage 60 ml. of 4.93% Oxypolygelatin was administered via the marginal ear vein. Samples were taken 5 hours later under the surgical conditions previously described. The pre- and post-dye blood samples were centrifuged, the cell volumes (26/100 and 21/100, respectively) were recorded, and the supernates were decanted. The plasma dilution factors (f) obtained from the expression

$$f = \frac{\text{ml. supernate}}{\text{ml. supernate} - \text{ml. oxalate}}$$

were found to be 1.37 and 1.34, and the corresponding hematocrit values were 32.5% and 26.3%. Optical densities of the supernates at 620 mμ were determined spectrophotometrically; the values observed were 66 and 390 for the pre- and post-dye plasma samples, respectively. Multiplication of each value by its appropriate dilution factor and subtraction of the products yielded the difference 429. This value was referred to the calibratiin curve (Fig. 2) extrapolated to zero time, and was found to correspond to a plasma volume of 151 ml. The blood

volume calculated from plasma volume and hematocrit was 205 ml.

The supernate of the dye-containing sample was diluted five-fold and 1-ml. aliquots were taken for the estimation of total protein and plasma protein by the Kjeldahl-Nessler and Folin-Ciocalteu micromethods described earlier. The Nessler analysis showed the sample to contain 0.76 mg. protein (N $\times$ 6.25), for which the corresponding corrected Folin reading was 303, yielding a specific Folin color value of 399 per mg. protein. Folin values similarly obtained were 79 for the Oxypolygelatin and 458 for the gelatin-free plasma proteins, the latter being obtained from the initial (zero time) hemorrhage samples. The ratio of gelatin to total protein was found graphically to be 0.16. The weight of circulating total protein, obtained by multiplying the amount of Nessler total protein-N by the dilution factors (1.34 and 5) and the plasma volume of 151 ml., was found to be 7.70 g. Therefore, the amount of gelatin present at 5 hours was 1.23 g. (7.70 $\times$ 0.16). The percent retention was obtained by dividing this value by the grams of gelatin injected (4.93% $\times$ 60 ml. = 2.96 g.), and found to be 42%.

3. *Results of Retention Tests*

The results of all the retention rests along with some of the pertinent physical properties are given in Table 6. The preparations which are listed represent those which were of the greatest importance in determining the optimum conditions for manufacturing procedures and the important batches which were made under pilot-plant conditions. Preparations 130-BI to 142-CIV were prepared in small amounts under laboratory conditions and have been previously discussed in Tables 1 and 2. Preparations 91 to 12-676AY were relatively large batches of OPG made under our supervision by Don Baxter, Inc. Preparations KI-3 to KII-5 were made by the Knox Gelatine Company. One polygelatin and one oxygelatin were also tested. The remaining six preparations were either untreated gelatins or gelatins which had been modified by heating. B20610–50 was supplied by the Upjohn Company, W186 by Wilson Laboratories, and P11–20 by Knox Gelatine

Company, and C-I, 78P, and 142-AI were prepared in our own laboratory.

TABLE 6

Retention Values for a Variety of Untreated and Treated Gelatin Preparations[1]

	Preparation	Source	Reagent ratio Glyoxal gelatin mg/g	Reagent ratio H_2O_2 gelatin mg/g	Osmotic pressure mm H_2O[2]	Relative viscosity 1.0% soln. *p*H 7.3, 37°C.	Average retention per cent 5 hr.	Average retention per cent 24 hr.
	Oxypolygelatins							
	91	Knox	25.0	31.0	97	1.23	42	13
	111	Knox	30.0	31.0	108	1.23	26	0
	113	Knox	25.0	31.0	104	1.32	32	0
	116	Knox	25.0	31.0	94	1.22	60	15
	117	Knox	25.0	31.0	92	1.28	34	7
	118	Knox	25.0	25.0	72	1.32	42	14
	11–115AY	Knox	15.0	15.0	122	1.20	35	23
	11–381AY	U-cop-co	15.0	15.0	98	1.35	39	26
	11–382Y	U-cop-co	15.0	25.0	113	1.27	40	15
	12–676AY	U-cop-co	10.0	10.0	87	1.26	38	16
	KI–3	Knox	25.0	30.0	..	1.23	48	23
	KII–3	Knox	12.5	15.0	..	1.25	41	25
	KII–5	Knox	12.5	22.5	..	1.21	25	7
1	130–BI	Knox	5.0	3.4	69	1.51	35	30
2	130–BIII	Knox	5.0	34.0	122	1.32	10	4
3	130–BIV	Knox	5.0	68.0	177	1.17	7	0
4	130–CI[3]	Knox	10.0	3.4	39	1.93	65	23
5	130–CII	Knox	10.0	17.0	75	1.53	42	33
6	130–CIII	Knox	10.0	34.0	86	1.31	44	24
7	130–DIII	Knox	15.0	34.0	91	1.36	27	0
8	142–BII	U-cop-co	5.0	3.3	65	1.36	70	23
9	142–BIII	U-cop-co	5.0	16.6	101	1.25	39	0
10	142–CII	U-cop-co	10.0	3.3	52	1.41	62	40
11	142–CIII	U-cop-co	10.0	16.6	92	1.28	40	17
12	142–CIV	U-cop-co	10.0	33.3	136	1.19	25	1
	142–CV	U-cop-co	10.0	66.6	190	1.15	7	0
	Polygelatin							
	142–C[3]	U-cop-co	10.0	0	41	1.39	39	24
	Oxygelatin							
	142–AIII	U-cop-co	0	16.6	89	1.25	20	0
	Gelatins							
	B20610–50	Upjohn	autoclaved		127	1.22	13	0
	C–I[3]	Knox	unheated		62	1.64	50	15
	78P[3]	Knox	autoclaved		78	1.43	30	15
	P11–20[3]	Knox	autoclaved		80	1.38	39	9
	142–AI[3]	U-cop-co	unheated		62	1.33	35	12
	W186	Wilson	autoclaved		106	1.24	0	0

[1]Other pertinent physical data will be found in tables presented in Part I.
[2]1.0% solutions at 37°C. and *p*H 7.0.
[3]Gel at room temperature, all other preparations were liquid. (Most OPG preparations were liquid at 10°C.)

The retention values are given for the 5-hour and the 24-hour period following transfusion. Each number is the average value for three to five rabbits. Although this small number of animals gave a relative wide spread of values, the general relationship among osmotic pressure, viscosity, and retention can be clearly seen. It will also be seen that retention values for OPG preparations tend to be somewhat higher than for unmodified gelatins with the same osmotic pressure. However, the most important fact which is shown is that OPG preparations which are liquid at room temperatures are well retained, whereas heat-degraded gelatins have very little retention value. There is no distinct correlation between osmotic pressure and retention of OPG preparations such as was found for heat degraded gelatins by Knoefel and Lehmann (10) and in our own studies. This would suggest a difference between preparations which is the result of a difference in molecular shape rather than in size.

Electrophoretic studies of serums from transfused animals indicate that the various OPG preparations had little or no effect on the normal pattern at the 5-hour and 24-hour periods of the standard retention test. Mixtures of various OPGs and rabbit serum made in vitro also showed no evidence of complexing. However, when rabbits were hemorrhaged of one-third of their blood volume and transfused with a similar volume of OPG solution at 24-hour intervals rather bizarre patterns began to appear after the third and fourth transfusion. For example, Figure 14 is a pattern, obtained under usual conditions, of serum from a rabbit 4 hours after the third hemorrhage and transfusion. Some complexing seems to have taken place, with the bulk of the globulins having a higher mobility than normal.

The chemical fractionation studies of OPG which were described in Part I clearly indicate heterogeneity with respect to molecular size. One might expect therefore that if sufficiently small molecules were present they would filter through the kidney and appear in the urine. Experiments made with preparation No. 91 showed that six hours after injection into hemorrhaged rabbits the OPG retained in the circulation had an average molecular weight of about 45,000 and the OPG

in catheterized urine was 15,000. In these experiments about 80% of the OPG was accounted for. Forty percent was in the circulation and 40 percent in the urine.

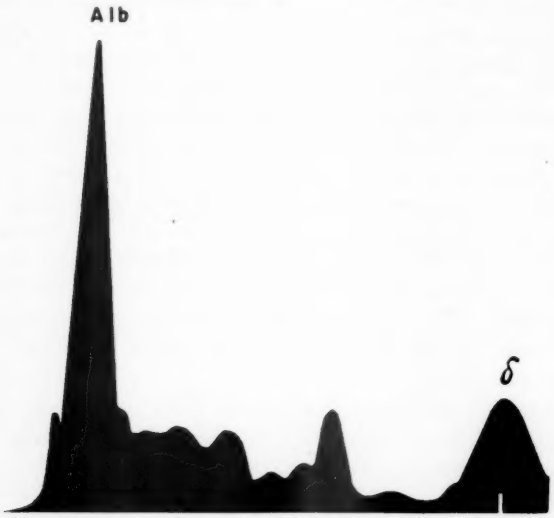

FIG. 14. The electrophoretic ascending pattern of plasma from a rabbit 4 hours after the third of three hemorrhages and transfusions made at twenty-four hour intervals. Electrophoretic conditions were 100 minutes at 20 ma. phosphate buffer at *p*H 7.3 and ionic strength of 0.10.

4. *Data Other Than Those Pertaining to Retention*

a) Effects on Protein and Fluid Balance in the Circulation.—Values for individual plasma volumes, hematocrits, and plasma protein concentrations varied considerably from one animal to another and hence cannot be averaged into one value for a given preparation. The results obtained were so near to the expected normal values that the presentation of detailed data is not justified here. In general, the plasma protein levels were considerably below normal at the 5-hour period but returned to about normal at 24 hours. The plasma volumes were within the limits of normal variation for the 5-hour and 24-hour periods. Hematocrit values reflected the loss of cells by hemorrhage and gave values of 20% to 35%. There was

no significant difference between the various OPGs and gelatins with respect to their effect on the blood and plasma volumes or the concentration of plasma proteins.

b) Restoration of Blood Pressure Following Hemorrhage.—To determine to what extent OPG could replace plasma proteins, a series of blood pressure studies were made on anesthetized rabbits from which approximately one-third of their blood volume was removed every hour and replaced with an equivalent volume of OPG plus washed erythrocytes from the preceding hemorrhage. Blood pressure measurements were made directly from the cannulated femoral artery, which was connected in the usual manner to a manometer and kymograph. Of fourteen rabbits tested, nine survived five or more hourly transfusions. The blood pressure of the survivors was approximately normal at the termination of the tests, with a slight increase in heart stroke volume of those receiving preparation 113. Unfortunately no electrophoretic studies were made of the plasmapheresed serums.

c) Toxicity.—Tests were made on each pilot-plant preparation for lethaal toxic activity in mice. One-ml. and 2.0-ml. amounts of OPG preparation were injected into the naudal vein of 10 mice for each amount. Of the earlier preparations No. 116 caused about 50% deaths in 1-ml. amounts and 100% in 2.0-ml. amounts; it showed no apparent toxicity for rabbits. Preparation No. 91, which was extensively used in the first studies, became lethal to mice in large amounts (2.0 ml.) after four months. All other preparations showed no lethal toxicity in 2.0-ml. amounts in 25–30 gm. mice.

d) Pyrogenicity.—Pyrogenicity tests were made on each pilot-plant preparation by Don Baxter, Inc., using standard methods. Since sufficient hydrogen peroxide was utilized in each procedure to destroy pyrogens the resulting bottled preparations were invariably pyrogen-free. Preparations 12676AY, 11381AY, and 11382AY tested pyrogen-free two years after manufacturing.

e) Antigenicity.—Antigenicity studies were made on all pilot-plant preparations. Tests were set up to determine if guinea pigs could be sensitized to the various preparations. Each preparation was injected intraperitoneally in 1.0-mg. and

100-mg. amounts. One group received one injection of the respective amounts, a second group received two injections and a third group received three injections. Animals were tested three to four weeks later for gross anaphylaxis by intravenous injection of about 20 mg. of material and for in vitro anaphylaxis on isolated intestinal strips. In no instance was antigenicity detected for the Knox or Wilson gelatins or for any of the OPGs made from them. During the course of the earlier studies antigenic gelatins were picked up, but we think that the antigenicity was probably the result of contaminating proteins other than gelatin.

5. *Clinical Studies*

These tests were primarily devised to determine human tolerance for intravenous injection of Oxypolygelatin and to study the effect of the injections on the blood and circulation. The patients were selected essentially at random, and in no instance was there an immediate demand for a transfusion except perhaps in the case diagnosed as amebiasis plus duodenal ulcer. The amount of OPG injected was at least 1 unit (500 ml.) per patient, and in one instance a total of 6 units was given over a period of a week.

The first tests, on eight patients at the Los Angeles County General Hospital, were performed under the direction of Dr. B. O. Raulston. The clinical data obtained from these patients are given in Table 7. The injection of 500 ml. of preparation 11–115AY was made intravenously over a period of one hour into each of the patients. The clinical data are not complete; they suffice, however, to indicate the absence of any gross incompatibility.

The second tests were made at White Memorial Hospital under the supervision of Dr. M. G. Levine. These tests were made on six patients, again selected at random. The clinical data obtained with this group are given in Tables 8 and 9. The methods of obtaining the blood clotting data and hematocrits were not reported to us, and these data are hence significant only for intercomparison. These tests indicate that OPG was well tolerated and produced no obvious undesirable

TABLE 7

Clinical Data from Patients Receiving One Unit (500 ml) of OPG 11–115AY

Patient	Time	Hematocrit	Sedimentation rate mm/hr.	Temperature	Respiration	Pulse	Blood pressure
H. M.	B	36	0	99.0	18	118	90/65
Arthritis	T	*	*	99.0	16	111	96/60
	1	*	*	98.8	14	94	88/60
	2	*	*	99.0	14	99	95/60
	3	*	*	*	14	102	90/60
T. J.	B	32	0	98.4	14	72	130/70
Peptic	T	*	*	98.4	14	63	140/70
Ulcer	1	*	*	98.4	14	66	135/70
	2	*	*	98.4	14	72	135/75
	3	*	*	98.4	14	60	128/66
J. E.	B	28	24	98.8	20	90	168/70
Pleural	T	*	*	*	24	90	168/68
Effusion	1	28	25	98.6	22	96	170/66
	2	*	*	*	22	90	154/70
	3	31	28	98.6	20	92	160/70
R. R.	B	32	16	98.0	24	69	122/78
Psychosis	T	*	*	98.2	22	66	124/70
	1	34	18	98.0	24	60	128/78
	2	*	*	98.4	24	72	122/76
	3	*	*	98.6	22	70	122/78
V. A.	B	37	12	98.4	24	87	118/74
Pneumonia	T	*	*	98.4	24	76	114/74
	1	32	4	98.4	24	84	116/72
	2	*	*	98.0	20	69	124/70
	3	25	14	98.6	22	86	122/70
J. W.	B	36	26	98.8	24	80	128/80
Pneumonia	T	*	*	*	24	72	112/75
	1	30	*	98.4	24	76	122/78
	2	*	*	98.4	24	66	116/70
	3	33	11	98.6	24	68	120/72
J. A.	B	31	24	97.8	24	69	106/66
Pneumonia	T	*	*	98.0	22	66	108/64
	1	29	11	98.0	24	80	104/60
	2	*	*	98.0	24	72	110/68
	3	*	*	98.0	22	70	112/64
H. H.	B	28	22	100.0	28	120	108/60
Delirium	T	*	*	99.0	24	108	100/60
Tremens	1	28	22	99.0	28	130	102/60
	2	*	*	99.4	24	116	106/62
	3	*	*	99.6	26	110	100/60

B—Before injection.
T—Termination of injection.
[1]Four hours after injection.
[2]Seven hours after injection.
[3]Twenty-five hours after injection.
*No determination made.

clinical response. It is especially significant that no marked changes occurred in hematocrit or sedimentation rate.

TABLE 8

Clinical Data from Patients Receiving One Intravenous Injection (500 ml) of OPG 1297AY

	Patient Z. J. Congestive heart failure			Patient G. M. Portal cirrhosis cardiac ascites			Patient V. M. Chronic pyelonephritis plus hypertensive heart failure			
Time	Before	After	24 hrs.	Before	After	24 hrs.	Before	After	24 hrs.	72 hrs.
Temperature	98.4	99.0	*	*	*	*	98.0	98.0	*	200/110
Respiration	16	20	*	24	24	*	16	16	*	99.0
Pulse	74	80	*	88	84	*	110	120	*	12
Bl. Pr.	160/70	180/90	*	150/75	160/95	*	200/125	200/110	6	120
Bleeding time	*	*	*	2′	10′	2′	*	*	*	*
Blood type	O	O	O	O	O	O	A	A	A	A
Coagulation time	*	*	*	3′40″	9′40″	9′53″	*	*	*	*
Prothrombin time	*	*	*	41%	39%	50%	*	*	*	*
Hematocrit	20	23	26	40	37	40	26	28	26	24
Sedimentation 15′	13	23	7	1	5	3	17	23	25	25
at 30′	22	34	17.5	3.5	12	8	30	32	34	34

*No data.

6. *Acknowledgments*

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TABLE 9

Clinical Data from Patients Injected with Two or More Units of OPG 12676AY

	Time hours	Temperature	Respiration	Pulse	Blood pressure	Blood type	Bleeding time (min.)	Coagulation time	Prothrombin time	Hematocrit	Sedimentation distance	
											15′	30′
L. P.	0[1]	98.6	20	80	102/78	A	2	6	78	19	7	16
Duodenal	1	99.4	22	92	100/70	OK	4	7	78	15	10	22
ulcer	4[2]	99.4	22	92	100/70	*	*	*	*	*	*	*
	5	100.2	24	78	104/70	OK	8.5	8.5	56	14	14	27
	29[3]	98.6	16	80	94/60	*	7	7	78	16	15	28
	30	98.6	20	80	94/60	OK	8.5	8.5	52	14	15	27
	34[4]	98.6	20	80	94/60	*	*	*	*	*	*	*
	35	99.4	16	88	100/70	OK	4	13.5	52	14	22	30
	59	*	*	*	*	OK	5	15	56	15	15	25
	83†	*	*	*	*	*	2.5	10	100	16	5	16
	84	*	*	*	*	*	4	10	100	18	4	11
	132	*	*	*	*	*	2	8	41	19	3	6
	180[5]	98.4	16	84	98/65	OK	2	5.5	100	19	3	8
	181	98.4	16	88	96/65	OK	3	7	100	18	3	11
	185[6]	98.4	16	88	96/65	*	2.5	7.5	74	16	10	19
	186	99.0	20	88	106/70	OK	2	9	45	17	6	14
F. B.	0[1]	98.2	*	*	*	A	2.5	13.2	87	33	0	0
Congestive	1	98.4	22	88	126/90	OK	3.0	5.3	47	31	0	0
heart failure	24[2]	98.0	22	84	120/78	OK	2.0	7.2	72	35	0	0
	25	98.0	20	88	110/74	OK	2.0	15.8	56	32	0	1.
	50	*	*	*	*	OK	3.3	7.8	*	28	0	0
G. P.	0[1]	*	20	80	110/90	B	14.7	28.5	36	13	30	33
Malignant	1	*	20	72	110/85	OK	4.0	12.0	33	12	33	35
hypertension	24	*	*	*	*	OK	11.0	9.6	40	12	30	36
	48[2]	97.0	16	76	144/100	OK	5.3	11.5	60	12	31	34
	49	*	16	76	144/104	OK	12.9	9.0	41	10	33	36
	73	*	*	*	*	OK	10.0	16.6	*	12	32	34

[1],[2]etc., injection of one unit of OPG.
†Whole blood (1,000 ml).
*No data.

variety of plasma substitute materials, including the first Oxypolygelatin preparations. These experiments were of great help in orienting us in the subsequent physiological tests. The studies made by Drs. T. Addis and Jean Oliver on the effect of OPG on kidney functions were of great value to us. We are also indebted to many of the members of the Committee on Medical Research for their encouragement and advice, and in particular to Dr. A. N. Richards. Many technical assistants were involved directly and indirectly in this program. The participation of all of these is greatly appreciated and in particular that of Mr. Bror Clark, Mary L. Sease, Louise S. Andersen, and Priscilla R. Feigen.

7. *General Summary*

The foregoing paper described the essential portions of the work on the modification of gelatin by treatment with a bifunctional condensing agent (glyoxal) and an oxidizing agent (hydrogen peroxide) for the purpose of obtaining a substance which could be used in emergencies as a substitute for blood plasma, serum, or serum derivatives. This product is referred to as Oxypolygelatin or OPG. Such a substance must have certain properties to meet obvious requirements, which have been discussed in the introduction of Part I (page 2). Oxypolygelatin has not been subjected to rigorous testing but the preliminary investigations which have been described in the foregoing discussions indicate that it is a very promising substitute. The data show that OPG is (1) tolerated by mice, rabbits, and dogs in large doses and by humans in at least practical amounts (no pathological studies have been made on tissues); (2) non-antigenic in guinea pigs or rabbits; (3) retained to the extent of 25% to 50% for 6 hours in hemorrhaged rabbits (the retention time can be controlled to some extent by altering preparation procedures); (4) osmotically active to about the same extent as, or somewhat greater than serum albumin; (5) digested in vitro by trypsin and pepsin (it disappears from the blood stream of rabbits in about 48 hours—no studies on possible storage in tissues have been made); (6) economical to prepare and can be made available in large amounts; (7) stable under a variety of normal conditions, such

as ordinary ranges in temperature and shaking (it can be administered without warming or other treatment under ordinary field conditions); (8) reproducible within practical ranges—further investigation is required to eliminate necessity for empirical control in manufacture. Oxypolygelatin preparations show a considerable degree of heterogeneity with respect to molecular size. Although this may or may not be desirable from a physiological standpoint, it certainly is undesirable from the standpoint of physical characterization and standardization of various preparations.

Much remains to be done on OPG, such as studies on the fundamental nature of the chemical and physical changes which take place during the modification of gelatin, the more precise regulation of molecular shape and size, and the investigation of its fate in the body. Other researchers on plasma substitutes in which Oxypolygelatin has been studied have been reported by Hamilton *et al.* (11, 12) and McCarthy and Parkins (13). However, sufficient groundwork has been done to provide a course for future work and suggest strongly that substances such as Oxypolygelatin should be seriously considered as practical substitutes for plasma in the restoration of circulation fluid under emergency conditions.

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TISSUE CULTURE STUDIES ON HUMAN SKIN

I. A Method of Evaluating the Toxicity of Certain Drugs Employed Locally on the Skin.*

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SKIN TOXICITY METHODS

Introduction

That certain drugs are more toxic when applied to the human skin than others is a well-known fact. However, the relative degree of toxicity is affected by many factors, such as the physiological state of the skin, the general condition of the patient and many other variables, including individual sensitivity to particular agents. There have been few methods to date by means of which compounds could be tested in the laboratory and accurate determination made as to the concentration of a given drug which could be expected to cause toxic symptoms when applied to the human skin. Previous to actual clinical trials, laboratory animals generally have been used for such experiments and while this method is useful, it is admittedly far from satisfactory in predicting the effect that a given drug will have upon human skin.

On the basis of published papers (Lewis, Pomerat, and Ezell, '49); Ulloa-Gregori, Blocker, Nowinski, and Pomerat, '50) and a large accumulation of newly acquired data, skin from grafting procedures performed on patients in good nutritional balance has been shown to produce epithelial sheets *in vitro*, in which a broad scope of cytological characteristics are made available for experimental analysis. In view of this fact, it was felt that this might prove to be a satisfactory method for evaluating the effects of drugs on the skin.

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The substances selected for study included salts known to be relatively nontoxic (sodium chloride) to the highly poisonous substance, bichloride of mercury. The group selected afforded an opportunity of determining the position of a new drug, SKFA,[1] which has anaesthetic and antipruritic properties in comparison with the local anaesthetics, Nupercaine and procaine, as well as with the antihistaminic agent, Thephorin.[2] In addition to these, boric acid and resorchinol provided additional substances with which the practicing dermatologist is thoroughly familiar and, therefore, which might prove useful in evaluating the method of studying toxicity with cells *in vitro*.

Materials and Methods

The use of tissue culture methods in the study of drug action, particularly for toxicity studies, has been recently reviewed (Pomerat, '51). It is pointed out that the techniques which have been developed have great potential value in pharmaceutical research due to their relatively low cost, rapid performance, stringency and reproducibility. Moreover, a great variety of genetically homogenous test objects are available. Ormsbee *et al.* ('47), as well as Biesele and his associates ('50, '51), have made good use of the method in screening compounds for their toxic effect upon tumor cells. Painter, Pomerat, and Ezell ('49), in a comparison of the toxicity of various clinically useful CNS stimulants and repressants, have established a simple procedure utilizing explants of chick embryo spinal cord and have reported a series of values which serve for comparative studies.

In the present report, tissue cultures were used to determine the smallest quantity of each chemical required to produce total inhibition of outgrowth from the various tissues examined. This value was designated as the minimal inhibitory dose (MID). In addition, the quantity of drug giving the slightest amount of injury as compared with untreated control cultures, or the least injurious dose (LID) was recorded. Since

[1]Now officially known as *Quotane (SKF)* and kindly supplied by Smith, Kline & French Laboratories.
[2]Kindly supplied by Hoffmann-La Roche, Inc.

screening operations with tissue culture methods are greatly facilitated with the use of test objects from embryos, these were employed for comparison with cultures of human skin.

Chick embryo tissues. The toxicity of each drug was tested with the use of explants of hearts from 7–9 days, spinal cords of the 9th day and spleens from 18-day chick embryos. Living cultures were examined daily, then fixed and stained for further study and preservation for records. Heart cultures were fixed after 48 hours of incubation in 10% formalin and stained with dilute Delafield's hematoxylin; spinal cords were fixed at 48 hours in formol bromide and stained with the Bodian method, while spleens were fixed at 24 hours in Helly-Zenker's and stained with hematoxylin-eosin-azure.

Four culture tests were made for each of the concentrations tested as well as for corresponding controls. Experiments were repeated on different days to reduce the possibility of errors due to variations in the test materials. Results obtained with chick embryo material in this report were based upon the study of more than 600 cultures.

Human epidermis. All drug toxicity tests on human skin were performed by one of us (E. E.) with the use of the following method. Skin was scrubbed with ward soap and rinsed twice with tap water. Rubbing alcohol was poured over the area and allowed to air dry. The skin was compressed between a sterile intestinal clamp whose blades had been covered with rubber tubing. Anaesthesia was allowed to develop in the clamped skin for 60 to 90 seconds. Thin slices of skin were removed by means of a sterilized razor blade and transferred to a Petri dish containing a few drops of Gey's balanced salt solution. The tissue was then cut into fragments approximately 2 mm. square for explanting. Explants were imbedded in a clot composed of 1 drop of plasma containing penicillin for a final concentration of 1,000 units per ml. of medium, and 1 drop of embryonic extract containing drug. Two drops of malignant ascitic fluid were added after the clot was firmly set. The cover glass on which the clot was made was then mounted on a hanging-drop slide, sealed with paraffin and incubated at 37° C. for 8 to 10 days.

Cultures were examined microscopically for growth daily or every other day. Readings on the 8th day were used for a final evaluation. A total of 255 skin cultures were employed for the data reported.

The test chemicals at various concentrations were mixed with the embryonic juice for incorporation into the medium. Titrations were repeated until values fell within a sufficiently narrow range of concentrations for the fixing of both the MID and the LID. Ratings of outgrowth from embryonic tissues followed the scheme outlined by Pomerat ('51).

Migration of epithelial cells from human skin usually began in 48 to 72 hours after the experiment was set up and continued until the 5th or 6th day. Outgrowth was graded by estimating the amount of the low power field it covered. A cellular sheet extending over one-fourth of the low power field was graded 1-plus; one covering the entire field was graded 4-plus, and intermediates between these two, a 2-plus covering one-half the field and 3-plus covering three-fourths of the field. Growth was usually stationary after the 6th day but cultures were read daily until the 8th day after being set up and final ratings were made on the 8th day.

Control experiments usually were found to have a uniform 4-plus outgrowth. Experiments in which controls did not give at least a 2-plus outgrowth were considered unsatisfactory and not taken into account in the summary of results.

No effort was made to select the skin used except for its gross thickness. All patients donating skin were considered to be in fair or better than fair nutritional balance at the time that the skin was taken.

Results

The minimal inhibitory dose (MID) and the least injurious dose (LID), expressed in gamma per ml. of culture media, for the eight drugs studied with the use of four different tissues are presented in Table I. These data are graphically represented in a logarithmic graph in figure 1. Results obtained for the human skin *in vitro* are shown separately in figure 2. The drugs are listed in order of increasing toxicity as based on the LID for the skin and appear in the following

order: sodium chloride, procaine, boric acid, resorcinol, SKF 538-A, Thephorin, Nupercaine and bichloride of mercury. The drugs ranged in toxicity from relatively nontoxic sodium chloride for which the MID falls between 3,900 and 7,800 gamma/ml., to the highly toxic bichloride of mercury which showed complete inhibition between 6.25 and 12.5 gamma/ml. and injury between 1.00 and 2.50 gamma/ml. of media. Sodium chloride was found to be two hundred times less toxic than the next two drugs, procaine and boric acid. Analysis of the group as a whole shows that the range of the LID for skin with all eight drugs falls into three groups: (1) sodium chloride, (2) procaine and boric acid, (3) resorcinol, SKF

TABLE I

Concentrations of Drugs in gammas per ml of Media

Drug	Tissue	MID	LID
Sodium Chloride	Chick Heart	6,250 – 12,500	1,560 – 3,120
	Cord	6,250 – 12,500	1,560 – 3,120
	Spleen	12,500 – 25,000	195 – 390
	Human Skin	3,900 – 7,800	2,500 – 3,900
Procaine	Chick Heart	1,000 – 1,250	80 – 160
	Cord	312 – 625	80 – 160
	Spleen	625 – 1,250	40 – 80
	Human Skin	1,000 – 2,500	12.5 – 25
Boric Acid	Chick Heart	6,250 – 12,500	780 – 1,560
	Cord	3,125 – 6,250	390 – 780
	Spleen	12,500 – 25,000	1,560 – 3,120
	Human Skin	312 – 625	12.5 – 25
SKF 538–A	Chick Heart	156 – 312	10 – 20
	Cord	20 – 40	5 – 10
	Spleen	312 – 625	10 – 20
	Human Skin	125 – 250	2.5 – 3.0
Resorcinol	Chick Heart	1,250 – 2,500	312 – 625
	Cord	625 – 1,250	80 – 160
	Spleen	2,500 – 5,000	160 – 320
	Human Skin	500 – 1,000	2.5 – 3.0
Thephorin	Chick Heart	125 – 250	60 – 125
	Cord	150 – 250	30 – 60
	Spleen	250 – 500	60 – 125
	Human Skin	25 – 30	2.0 – 2.5
Nupercaine	Chick Heart	30 – 60	2.0 – 4.0
	Cord	30 – 60	10 – 20
	Spleen	156 – 625	30 – 60
	Human Skin	25 – 30	1.25 – 2.5
Bichloride of Mercury	Chick Heart	6.25 – 12.5	0.8 – 1.6
	Cord	6.25 – 12.5	0.4 – 0.8
	Spleen	50 – 100	1.6 – 3.2
	Human Skin	6.25 – 12.5	1.00 – 2.5

538-A, Thephorin, Nupercaine and bichloride of mercury. With this grouping, it is seen that sodium chloride produces injury in the range of 2,500 to 4,000 gamma/ml. of culture medium; procaine and boric acid produce injury between 12.5 and 25 gamma/ml., while the remaining five drugs will cause demonstrable injury at concentrations as low as 1.00 to 3.00 gamma/ml. of media.

Complete inhibition (MID) of all outgrowth of the skin by the several drugs varies from a dose as small as six times the injurious dose in the case of bichloride of mercury to approximately twenty-five times the LID for boric acid. Arrangement of the drugs in order of increasing toxicity as based on the MID gives the following: (1) sodium chloride, (2) procaine, (3) resorcinol, (4) boric acid, (5) SKF 538-A, (6) Thephorin, (7) Nupercaine and (8) bichloride of mercury. Thus, it is evident that boric acid, though significantly less toxic than the five drugs listed after it so far as injury is concerned, is

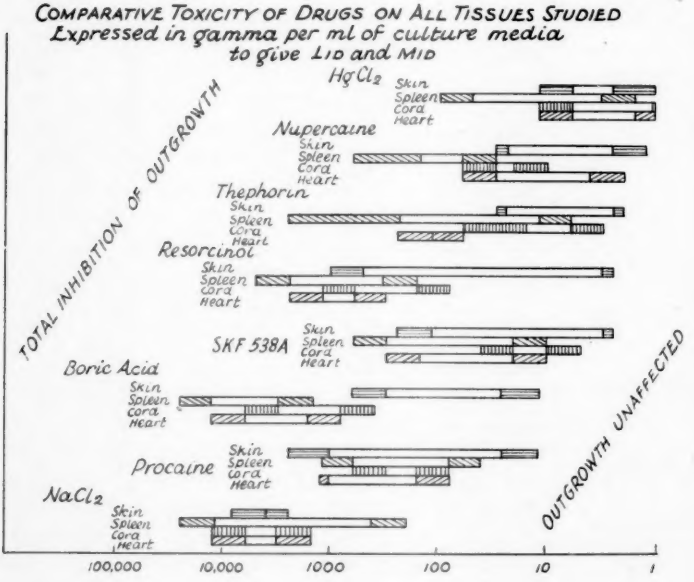

FIG. 1

slightly more toxic than resorcinol in that it produces complete inhibition of outgrowth at approximately two-thirds the MID of resorcinol. It is also interesting to note that sodium chloride, though nontoxic for the skin outgrowth at a concentration between 2,500 and 3,900 gamma/ml., causes complete inhibition of all outgrowth at twice this concentration, or 3,900 to 7,800 gamma/ml. of culture medium.

Comparison of embryonic tissues and human skin reveals that in all instances the skin is more sensitive than the embryonic tissues. Moreover, the embryonic chick tissues follow a regular pattern in which the cord is most sensitive, the heart second and the spleen least sensitive, with one exception; chick heart is less sensitive to procaine than is chick spleen.

The range of concentration presented as the MID and LID varies widely in some cases, while in others it is relatively small. This fact becomes more understandable when one investigates the drugs involved. In the case of sodium chloride,

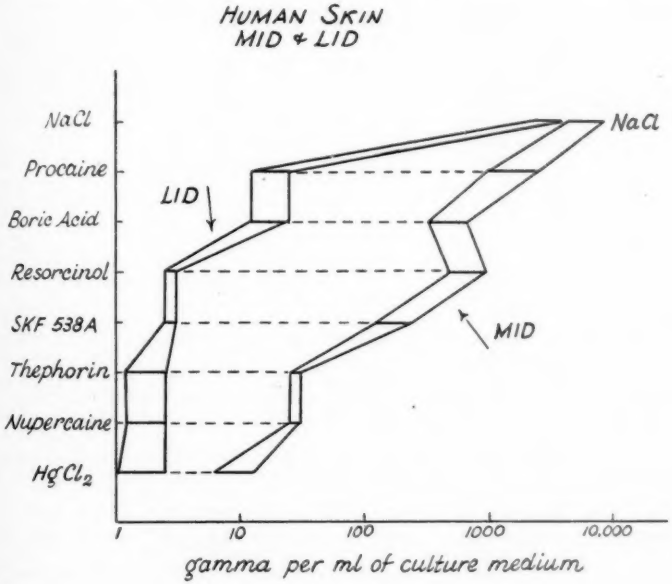

FIG. 2

for example, the MID is between 3,900 and 7,800 gamma/ml. With this particular drug complete inhibition of outgrowth occurred at 7,800 gamma/ml. of culture medium, while a 1-plus outgrowth occurred at 3,900 gamma/ml. Between these two concentrations, various degrees of cellular emigration occurred. However, unless the outgrowth attained a 1-plus value it was considered negative and, consequently, the range remains wide. With a more refined technique of measuring outgrowth, it would be possible to narrow these MID and LID ranges. In the case of the more toxic drugs in which complete inhibition and injury occur at very low concentrations, it is possible to narrow the range of concentrations markedly.

TABLE II

Molal Concentrations of Drugs per ml of Media

Drug	Tissue	MID	LID
Sodium Chloride (M.W. 58.45)	Chick Heart	$2.14 \times 10^{-1} - 1.07 \times 10^{-1}$	$5.35 \times 10^{-2} - 2.67 \times 10^{-2}$
	Cord	$2.14 \times 10^{-1} - 1.07 \times 10^{-1}$	$5.35 \times 10^{-2} - 2.67 \times 10^{-2}$
	Spleen	$2.14 \times 10^{-1} - 1.07 \times 10^{-1}$	$6.68 \times 10^{-3} - 3.34 \times 10^{-3}$
	Human Skin	$1.34 \times 10^{-1} - 6.68 \times 10^{-2}$	$6.68 \times 10^{-2} - 4.27 \times 10^{-2}$
Procaine (M.W. 236.17)	Chick Heart	$5.29 \times 10^{-3} - 4.23 \times 10^{-3}$	$6.61 \times 10^{-4} - 3.31 \times 10^{-4}$
	Cord	$2.65 \times 10^{-3} - 1.32 \times 10^{-3}$	$3.31 \times 10^{-4} - 1.69 \times 10^{-4}$
	Spleen	$5.29 \times 10^{-3} - 2.64 \times 10^{-3}$	$3.31 \times 10^{-4} - 1.69 \times 10^{-4}$
	Human Skin	$1.06 \times 10^{-2} - 4.23 \times 10^{-3}$	$1.06 \times 10^{-4} - 5.29 \times 10^{-5}$
Boric Acid (M.W. 61.84)	Chick Heart	$2.02 \times 10^{-1} - 1.01 \times 10^{-1}$	$2.53 \times 10^{-2} - 1.26 \times 10^{-2}$
	Cord	$1.01 \times 10^{-1} - 5.05 \times 10^{-2}$	$1.26 \times 10^{-2} - 6.32 \times 10^{-3}$
	Spleen	$2.02 \times 10^{-1} - 1.01 \times 10^{-1}$	$5.05 \times 10^{-2} - 2.53 \times 10^{-2}$
	Human Skin	$1.01 \times 10^{-2} - 5.05 \times 10^{-3}$	$3.66 \times 10^{-4} - 1.83 \times 10^{-4}$
Resorcinol (M.W. 110.11)	Chick Heart	$2.27 \times 10^{-2} - 1.14 \times 10^{-2}$	$5.68 \times 10^{-3} - 2.84 \times 10^{-3}$
	Cord	$1.14 \times 10^{-2} - 5.68 \times 10^{-3}$	$1.42 \times 10^{-3} - 7.09 \times 10^{-4}$
	Spleen	$4.55 \times 10^{-2} - 2.27 \times 10^{-2}$	$2.84 \times 10^{-3} - 1.42 \times 10^{-3}$
	Human Skin	$9.08 \times 10^{-3} - 4.54 \times 10^{-3}$	$2.84 \times 10^{-5} - 2.27 \times 10^{-5}$
Thephorin (M.W. 263.37)	Chick Heart	$9.49 \times 10^{-4} - 5.93 \times 10^{-4}$	$4.74 \times 10^{-4} - 2.37 \times 10^{-4}$
	Cord	$9.49 \times 10^{-4} - 5.93 \times 10^{-4}$	Less than 1.19×10^{-4}
	Spleen	$9.49 \times 10^{-3} - 9.49 \times 10^{-4}$	$4.74 \times 10^{-4} - 2.37 \times 10^{-4}$
	Human Skin	$1.19 \times 10^{-4} - 9.49 \times 10^{-5}$	$9.49 \times 10^{-6} - 4.75 \times 10^{-6}$
SKF 538-A (M.W. 308.84)	Chick Heart	$1.01 \times 10^{-3} - 5.05 \times 10^{-4}$	$6.31 \times 10^{-5} - 3.15 \times 10^{-5}$
	Cord	$1.26 \times 10^{-4} - 6.31 \times 10^{-5}$	$6.31 \times 10^{-5} - 3.15 \times 10^{-5}$
	Spleen	$2.02 \times 10^{-3} - 1.01 \times 10^{-3}$	$6.31 \times 10^{-5} - 3.15 \times 10^{-5}$
	Human Skin	$8.09 \times 10^{-4} - 4.04 \times 10^{-4}$	$8.09 \times 10^{-6} - 4.05 \times 10^{-6}$
Nupercaine (M.W. 379.72)	Chick Heart	$1.65 \times 10^{-4} - 8.23 \times 10^{-5}$	$1.03 \times 10^{-5} - 5.14 \times 10^{-6}$
	Cord	$1.65 \times 10^{-4} - 8.23 \times 10^{-5}$	$5.14 \times 10^{-5} - 2.57 \times 10^{-5}$
	Spleen	$1.65 \times 10^{-3} - 4.11 \times 10^{-4}$	$1.65 \times 10^{-4} - 8.23 \times 10^{-5}$
	Human Skin	$8.23 \times 10^{-5} - 6.58 \times 10^{-5}$	$6.58 \times 10^{-6} - 3.29 \times 10^{-6}$
Bichloride of Mercury (M.W. 271.51)	Chick Heart	$4.60 \times 10^{-5} - 2.30 \times 10^{-5}$	$5.75 \times 10^{-6} - 2.88 \times 10^{-6}$
	Cord	$4.60 \times 10^{-5} - 2.30 \times 10^{-5}$	$2.88 \times 10^{-6} - 1.44 \times 10^{-6}$
	Spleen	$1.84 \times 10^{-4} - 9.20 \times 10^{-5}$	$1.15 \times 10^{-5} - 5.75 \times 10^{-6}$
	Human Skin	$4.60 \times 10^{-5} - 2.30 \times 10^{-5}$	$9.21 \times 10^{-6} - 4.60 \times 10^{-6}$

Table II indicates the MID and LID of the drugs studied in terms of their molal concentrations. The same material as it relates to human skin is presented graphically in figure 3.

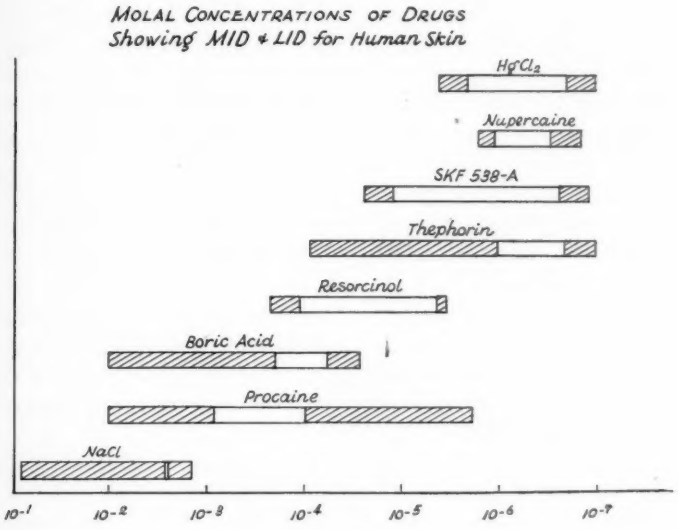

FIG. 3

Analysis of these results indicates that toxic dosages expressed as molal concentrations assume the same general relations to each other as those reported in terms of gamma/ml. of medium. Individual variations show that resorcinol with a LID of 2.84×10^{-5} to 2.27×10^{-5} mils per ml. of culture medium is less toxic than SKF 538-A, Thephorin and Nupercaine. These drugs present practically the same LID when compared on the basis of gammas/ml. of culture medium. It is also evident that procaine is slightly more toxic than boric acid on the basis of molal concentrations.

Data obtained for the toxicity (MID) to the chick spinal cord of the chemicals used in this study can be compared with values obtained by Painter, Pomerat, and Ezell ('49) for various stimulants and depressants of the central nervous system. Bichloride of mercury is approximately twice as

toxic to nervous tissue as is strychnine sulfate. The two local anaesthetics, procaine and Nupercaine, when compared with cocaine indicate that procaine is only one-half as toxic as cocaine, whereas Nupercaine is approximately four times as toxic as cocaine for nervous tissue. Boric acid and sodium chloride were found to be more injurious to nerve tissue than sodium bromide or nicotinamide. Thephorin, the antihistaminic studied, indicated a tixicity for nerve tissue in the same general range as ephedrine and was less toxic than epinephrine.

Summary

1. Studies of human epithelium *in vitro* indicate that this tissue is a satisfactory test object for the evaluation of the relative toxicity of drugs.

2. The minimal inhibitory dose (MID) and least injurious dose (LID) of eight drugs employed in dermatological practice were determined with the use of tissue cultures of human skin as well as with spinal cord, heart and spleen fragments from chick embryos.

3. Listed in order of increasing toxicity as based on the LID for human skin, the eight drugs included sodium chloride, procaine, boric acid, resorcinol, SKF 538-A, Thephorin, Nupercaine and bichloride of mercury.

4. Comparative results indicate that for both embryonic chick tissues and human epithelium, sodium chloride was the least toxic and bichloride of mercury the most toxic of the drugs which were studied.

5. Embryonic chick cord is more sensitive to the drugs studied than either chick heart or spleen.

6. In general, epidermal cells from human skin were more sensitive to the drugs studied than were embryonic chick tissues.

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WHAT IS MENTAL ILLNESS*

J. R. Ewalt

With the vast increase of public interest in psychiatry and general problems of mental health and with the smaller but still healthy increase of interest in psychiatry by the medical schools and the medical profession in general, it becomes necessary for psychiatrists to stop and take stock of their professional situation. We find psychiatrists of excellent repute writing on subjects ranging far afield from the rigid confines of the medical specialty in which they are trained. Most issues of daily newspapers and magazines contain some articles by either real or self-delegated psychiatrists, clinical psychologists or other persons purporting to have some knowledge of the functioning of the human personality. For knowledgeable people to range afield from their own particular orbit of activity is a healthy sign. General Eisenhower has defined education as the preparing of individuals to be effective citizens in a free world. Certainly as educated persons, psychiatrists have responsibilities as citizens.

It is healthy for phychiatrists to look upon their activity in two distinct categories, though in practice they may be interrelated and inseparable. First, we are physicians, we are trained to care for patients who suffer from mental illness, to guide in programs designed to prevent illness and to conduct researches into the cause of illness. Second, we are educated citizens and should take a broad interest in the activities of the culture in which we live. We should remember that in the management of the situations in the first category we are the leaders and should take the pre-eminent role in these medical matters. We should likewise remember that when we start talking or working in spheres outside of our own particular specialty that we are no more and perhaps no less well prepared than some of our other educated colleagues for understanding, interpreting and carrying out progressive functions

*Address delivered at V. A. Hospital, Little Rock, Arkansas, January 30, 1951. From The University of Texas Post-Graduate Medical School, Houston.

in the broader spheres of sociology, economics, statesmanship and politics. Attempts to "analyze and treat" situations in these spheres from a purely psychiatric orientation is ridiculous.

Most of you would agree to the above-mentioned principles. The difficulty comes in deciding where mental illness leaves off and where one begins to function as an ordinary citizen with some special brand of knowledge.

What then comprises "mental illness"? Is it the bedwetting child, is it the hallucinating woman, does it include stomach ulcers, does it include the interracial tension in certain sections of our cities, does it include the international tension manifested at the United Nations' Councils, is it the drunken driver, is it the speed-mad driver, skillful though he may be? Perhaps, as a starting point, we could all agree that it goes beyond the Shakespearean definition of "is but to be mad."

Freud describes neuroses and psychoses as follows: "*Neurosis* is the result of a conflict between the *ego and its id*, whereas *psychosis* is the analogous outcome of a similar disturbance in the relation between the *ego* and its environment (outer world)."

The symptoms of a neurosis if defined as conflict between the ego and the id would then include many types of people using reasonably acceptable modes of adjustment. Would the person who abides by the biblical directive, "Blessed are the meek, for they shall inherit the earth," by such manifestations of passivity compensated for by hopes of a reward guaranteed by faith alone be looked upon as stations of a neurosis, are persons epitomized by Edna St. Vincent Milet's little couplet "My candle burns at both its ends, It will not last the night, But ah, my foes and oh my friends, it makes a lovely light," to be considered as suffering from a neurosis?

Goldstein speaks of "catastrophic behavior" in a situation in which the patient is confronted with a sense of failure on a level which is unacceptable to him, this in contrast to the ordered behavior that a person has in situations with which he can cope.

Lin Yutang in the "Importance of Living" has expressed it—"I think that, from a biological standpoint, human life almost reads like a poem. It has its own rhythm and beat, its internal

cycles of growth and decay. It begins with innocent childhood, followed by awkward adolescence trying awkwardly to adapt itself to mature society, with its young passions and follies, its ideals and ambitions; then it reaches a manhood of intense activities, profiting from experience and learning more about society and human nature; at middle age there is a slight easing of tension, a mellowing of character like the ripening of fruit or the mellowing of good wine, and the gradually acquiring of a more tolerant, more cynical and at the same time a kindlier view of life; then in the sunset of our life, the endocrine glands decrease their activity, and if we have a true philosophy of old age and have ordered our life at and according to it, it is for us the age of peace and security and leasure and contentment; finally life flickers out and one goes into the eternal sleep, never to wake up again.

"We should be able to sense the beauty of this rhythm of life, to appreciate as we do grand symphonies its main theme, its strains of conflict and the final resolution. The movements of these cycles are very much the same in a normal life, but the music must be provided by the individual himself. In some souls the discordant note becomes harsher and harsher and finally overwhelms or submerges the main melody. Sometimes the discordant note gains so much power that the music can no longer go on, and the individual shoots himself with a pistol or jumps into a river. But that is because his original *leit-motief* has been hopelessly overshadowed through the lack of a good self-education."

The general context of all these proposals would suggest that a person may be said to be mentally ill when situations with which he is confronted so far exceed his adaptive capacities that he must resort to distortion of body function, to distortion of the facts of the real world about him, or perhaps in a broader sense when he resorts to behavior that is detrimental to persons in his environment, this protective behavior being necessary if he is to escape flooding of his consciousness with anxiety or repressed material unacceptable to him. Thus, we would come to understand mental illness as being a type of adjustment that an individual makes to certain demands made

upon him. The adjustment, unsatisfactory though it may be, is the best possible one under the circumstances.

We call it mental illness because the resultant total behavior pattern does not conform to the standards of the time and the situation. For example, a patient in clinic in dungarees and sans shave would be accepted, but if I came here so dressed you would think I was psychotic. If I came to a picnic in full dress you would think that odd. If I came here drunk, you would say "alcoholic," but if I delivered a temperance lecture you would think that even more peculiar. Thus, we are labeled ill, a genius, brilliant, or a fool, depending upon how well our ego can adapt our means of satisfying the basic drives to the demands of our particular culture and time.

If we are to understand mental illness we must, therefore, understand what goes to make up the person. That is, we must understand his basic needs, and his capacities to satisfy these needs in the environment in which he finds himself. We must study carefully the interaction between the patient and the environment if we are to have an all out understanding of the facets of his illness. A study of the basic needs of man, sometimes dubbed the "instincts," has been the preoccupation of philosophers, religionists, scientists and psychiatrists for many generations.

Confucius wrote that the basic needs of man were for food, drink and women. Goldstein has stated that the basic drive in all humans was for self-realization. By this he apparently means the need to find oneself in a satisfactory relationship with the other persons in the environment, and the ability to live in a satisfactory manner in this situation. Of course, the basis of modern psychology and its understanding of human function rests in the works of Sigmund Freud.

It would be superfluous to repeat here the emphasis in Freud's ultimate teachings of the basic urges of the sexual and death or aggressive instincts. We would stress, however, that the understanding of a patient's distortion of behavior in the present and now is not to be fully realized by the investigations of the vicissitudes of his ego development as though they existed in some Erlenmeyer flask in the sterile room of a tissue culture laboratory. The investigation of these earlier

developmental phases are essential if one is to understand what goes to make the individual that is reacting to the situation as of now. On the other hand, it would be equally, or perhaps even more serious to ignore the actual situation in which he is at present reacting. It should always be remembered that the earlier material is essential only in that one cannot understand the individual nor his reactions to his present situation that hurts unless you have an understanding of these earlier facets of his development.

The importance of a consideration of the present demands of reality has been emphasized by practically all profound writers on the subject, but is often neglected or overlooked by the neophyte in the field. Mark Twain once said, "Circumstances make man, not man circumstances." Freud himself has expressed it as clearly as any: "Or to express it yet another way, neurosis does not deny the existence of reality, it merely tries to ignore it; phychosis denies it and tries to substitute something else for it. A reaction which combines features of both of these is the one which we call normal or healthy; it denies reality as little as neurosis but then, like a psychosis, it is concerned with affecting a change in it. This expedient normal attitude leads naturally to some active achievement in the outer world and is not content like a psychosis with establishing the alteration within itself; it is no longer autoplastic, but alloplastic."

Fundamental in the understanding of the interaction of these two activities is an understanding of what Franz Alexander has called "the power of regressive forces." He points out that human beings in general have a tendency to refuse to accept changes, especially those which require a greater independence and expenditure of energy and an inclination to revert to earlier behavior patterns, whenever a new situation requires a new adjustment. He emphasizes that regression does not mean an exact repetition of older methods of behavior or older attitudes, but merely a coloring of present behavior by earlier attitudes. Thus the examples he uses are: "Drinking alcohol is not identical with suckling; cigar smoking or chewing is not the same as thumbsucking; and the street phobia is not precisely the old fear and insecurity of the child when he is first

called upon to do an errand by himself." He goes on to state, however, that they are attempts to revive earlier situations which will enable the person to in some manner avoid the unpleasant demands of the present.

Balanced against these regressive trends with their tendency to economize on nature are the basic urges and drives of man to create. This is manifest in the sexual sphere, in the growth and development of children, man's desire to acquire new mastery over his environment, new spheres of knowledge, etc. The irrational competition between people probably does not fall within this particular sphere of activity. A broad concept of sociologic factors is necessary in order to fully understand the interreaction between these two forces. It is also indicative of the degree which technicological factors have expanded beyond the concept of sociologic factors in the interrelationships of people.

The reasons for the neglect of religion and philosophy and the concentration on television and jet motors is a matter for study of our colleagues in the allied discipline. Psychologically speaking, it certainly is a manifestation of the earlier mentioned regressive tendency in man. The desire to lead an increasingly parasitic existence where a man works and sweats and if he avoids dying of coronaries, can retire into a thermostatically and automatically controlled old age, where the only efforts necessary to living are to digest his own food and to pass his own excretions, and even here science has introjected enzymes to artificially aid him with his digestion and enema tubes in order to speed up his evacuation with less effort to himself. All of these factors present individually and as a group in our culture have an important facet in the development of mental illness and any consideration of the diagnosis and treatment of such disorders.

Thus, so-called mental illness may stem from any one of several causes. First, and the simplest situation, would be that the environment was so difficult that no amount of adaptive capacity would make a person get along entirely well. An example would be the unfortunate navigator of a constellation plane over the Atlantic, who was precipitated out the navigation dome by pressurized air within the cabin. Nature,

unfortunately, forgot to equip him with wings in order that he might soar downward and in the second place had he done so, having landed in the water in the middle of the Atlantic it is doubtful that he could have remained afloat long enough to be rescued, inasmuch as he lacked fins or sufficient coating of blubber to preserve him from the temperature. One might term this environmental difficulty, a type of constitutional defect reaction if one wished to speak facetiously.

A different category, but closely akin to this, is the person precipitated into this rather difficult world in which we live, who has been by nature under-equipped in terms of cortical tissue in order that he might understand at least some of the implications of the things that go on about him. Various combinations of difficult situations and varieties of basic capacities go into explaining many other types of illness. If persons in their emotional growth fail to develop sufficient ego strength to enable them to cope with an ordinary environment, they inevitably succumb to some type of mental illness unless the environment about them is extraordinary in terms of protective and ego-supporting potentials.

We have other persons who, in the process of growth build a strong ego or a great capacity for integrating their needs with the demands of the environment, but who, in the course of their activities meet with an extraordinary situation. These patients, faced with failure, retreat to earlier and more successful types of adjustment, usually referred to as symptoms, or if you prefer, regressive phenomena.

We also have the person who, during the phases of his ego development gets along quite well and is able to cope with the ordinary environment, but who due to sclerosis, the aging process, syphilis, tumor, trauma, or other cortical depleting processes begins to lose some of the adaptive functions which he has developed and then he too begins to have difficulty in conforming to the demands of his environment as he attempts to satisfy his basic needs.

Patients in this latter category are referred to as organic. Those in the former category are referred to as functional. Actually, there is little difference except in the facet of their ego, which lacks the ability to receive the demands of the

environment, take cognizance of the needs of the organism and to work out a solution that is satisfactory. In the so-called organic case there is an actual loss of cortical functions which reduce the patient's capacity to function. Abilities formerly present and relied upon in establishing his behavior patterns are gone. To survive he must change his behavior to conform to the new top limit of his capacities.

In the so-called functional cases this process is a little more complicated, but the end results are essentially the same. In these categories the person by reason of experiences in the process of taking from his environment those things necessary for his continued existence comes upon situations which threaten him by reason of old conditioning or exceed his capacities by reason of previous imperfections in development.

In either instance the patient is now in a situation which is no longer tenable, so far as he is concerned, and it is necessary for him to begin to simplify the environment. In some instances the situation is sufficiently superficial or sufficiently controllable so far as environmental manipulation is concerned that relatively simple procedures result in successful mastering of the situation.

For example, a person in a job which is incompatible or which makes him very apprehensive, may quit, and take a job making fewer demands upon him. In this case, we may say that he has worked out a type of solution which is in general satisfactory to him and no further activities are required on his part.

In the case of the organic patient, if he has reached a certain specified chronological age, perhaps he can retire, and thus reduce the demands upon him and in this manner simplify his environment and solve his problems. Other patients find that such solutions are either unacceptable to them or in some instances unavailable. This is particularly true where the difficulty involves personal attitudes of the patient and the environment as such does not lend itself readily to manipulation or significant change. In these instances the patient will resort to what we call nervous or mental symptoms. In effect he is still attempting to simplify his environment but

is using a different method, which results in the formation of symptoms such as faints, pains, amnesia, periods of anxiety.

The selection of symptoms will depend upon the individual, his physical makeup and the situation in which he finds himself. In some the development of such symptoms solves the problem and they go through life as chronic neurotics. Others, more fortunate, receive therapy and if successful grow in ego strength so they can cope with the aforementioned difficulty. In still others, time takes care of the environment and precipitates the patient into situations more nearly satisfactory and with some reluctance he may give up his illness.

Some patients find that so-called neurotic symptoms will not provide a satisfactory solution. They are still threatened and the situation continues to stir up anxieties and insecurities, threatening what Goldstein has called a "catastrophic situation" and which the analysts call "flooding the consciousness with anxiety."

Whichever term you prefer the patient is sorely threatened and if he is to exist must bring about more drastic changes. The patient must then begin to distort and misinterpret the environment, bending it into shapes more acceptable to him. We commonly call such a patient "psychotic." We say that the patient has regressed and ignores the demands of reality. Actually the patient has regressed, but to state that he ignores the demands of reality is taking a very superficial view of it. Actually, he is so much aware of the demands of reality and what they mean to him both consciously and unconsciously that he finds it necessary to distort them into a form more satisfactory to him, or at least into a form he is able to cope with using the particular resources and abilities available to him at the given time.

The importance of this consideration of what we mean by "mental illness" is by now rather obvious. In the first place it tends to highlight the areas in which we have little or no information. Therefore it serves as a more accurate guide to future investigative activities. In the second place it enables us to plan therapeutic programs more intelligently and make a more efficient use of our colleagues in the allied disciplines. In the third place, when we understand what happens to a

patient when he develops mental illness, it gives us many guides for our activities in the preventive field. It enables us to give more intelligent guidance to our colleagues and co-workers who must play the major role in the actual mechanics of the prevention of possible mental illness.

Thus our understanding that general paresis is due to a patient's failure to adapt himself to an environment because of the spirochets damage to the cortex makes treatment intelligently planned go along the lines of (1) combating the actual infection, and (2) decreasing his responsibilities and otherwise simplifying his environment so he can cope with it in terms of the resources he has left and (3) enabling the patient, through psychotherapy, to more readily accept and understand what has gone on in order that he may more nearly adapt himself to his new position in life. Understanding the infectious origin of this illness also makes prevention possible by early treatment and co-operation with the social hygienist and other anti-venereal disease campaigns to cut down the overall incidence of infection.

The understanding of the meaning of the functional disorders, both neurotic and psychotic, makes our co-operation and work with the parents and teachers who work with youngsters during the formative years seem important facets of our activities. Certainly time spent in indoctrinating pediatricians and the general practitioners who first advise mothers on the care of the children and working with schoolteachers, is all time constructively spent in prevention.

Treatment of these patients must also be multi-faceted. First, attempts to make the person grow in his abilities to adjust to the world must be carried on by psychotherapy on either a depth level or on a brief or segmental level, which is oriented to some effective form of psychology. It also enables one to carry out another facet of the program, and that is an understanding of the dynamics of the group in the environment and its manipulation, in order that the demands made upon the individual may also be brought more within his capacities to adjust.

In our public, non-medical functions this knowledge of man's nature is of value. Franz Alexander has stated "The main

defect of the social philosophies of the past—both the dream-like utopias and also the sober economic theories—has been their ignorance of the nature of human personality. Social life is lived by human beings, and the dynamic principals of social life are the direct expression of man's psychological makeup. Any social theory not based on correct psychology is an abstraction with limited affiliation to actual social phenomena."

Alexander Wolcott makes similar remarks: "For the architects of any Utopia are always inconvenienced by the discovery (usually at the last minute) that the human race is not, as at present, matured, equipped for the exacting role forced upon it by citizenship in Utopia."

OBSERVATIONS ON THE TOXICITY OF 3,3-DIETHYL-2,4-DIKETOTETRAHYDROPYRIDINE (PRESIDON)*

MARTHELLA FRANTZ AND C. M. POMERAT†

Pharmacological study of the hypnotic sedative 3,3-diethyl-2,4-diketotetrahydropyridine (Presidon) indicated that it produced no untoward reactions in experimental animals nor in man (*cf.* Koppanyi, Herwick, Linegar, and Foster, '40; Freed, '47; Polatin, Hirschberg, Rubin, and Pirk, '47; Polatin and Horwitz, '47; Wilbrandt and Jaeger, '48; Parsonnet *et al.*, '48; Becker, Fabing, and Hawkins, '49). Its clinical use for about eight years in Europe failed to suggest the capacity of Presidon to produce leucopenia and agranulocytosis as reported recently in a few instances by workers in the United States (Rose and Marchand, '49; Tyson, '49; Ehrlich and Sussman, '49; Covner and Halpern, '50).

Evidence has been presented to indicate definite allergic response in a patient in which the bone marrow under the test doses showed an initial vigorous stimulation along with a tendency to a marked drop in number of circulating leucocytes.

Pharmacologists have become increasingly aware of the inadequacy of existing techniques for the study of the depressing or irritating action of new synthetic drugs on haemopoiesis. A special subcommittee for the study of agranulocytosis has been organized within the Medical Section of the American Drug Manufacturers Association. The present study was undertaken with the hope that suggestions for new methods might arise which would prove useful in detecting the toxicity of drugs on blood cells with the use of tissue cultures of human bone marrow or of the buffy coat. Presidon appeared to be precisely the type of test object which might serve this purpose.

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Since the literature failed to reveal the results of systematic observations on the circulating blood of experimental animals having received chronic high doses of Presidon, it was decided that this procedure should constitute a base line for *in vitro* studies.

I. Toxicity Tests on Mice

Materials and Methods

All blood counts, as well as the responsibility for the diet and care of experimental animals, were carried out by one of us (M. F.). All animals used were of the CBA stock of mice, which is well beyond the 25th inbred generation and therefore homogeneous. A total of 46 animals were employed. These consisted of four different age groups: 185, 135, 90 and 50 days old at the time when the experiments were begun. Each group, designated as I, II, III and IV, respectively, was divided into three series of approximately equal numbers of male and female animals which received 200 gamma of Presidon[1] per gram of body weight per day for 30 days (1) subcutaneously, or (2) intravenously, or (3) no Presidon (CONTROLS).

Blood counts were performed on four successive days before beginning the experiments and for two consecutive days after the treatment period (30 days later). Counts were done only on the groups of animals receiving Presidon, using the pretreatment counts as control values. Heart blood was obtained by cardiac puncture without anaesthesia. For obtaining tail blood the following procedure was followed: the mouse was "trapped" in a square of wire screening molded so that the four sides could be fixed to a small board by tacks. The board was then suspended from a ring-stand so that the tail could be completely immersed in a bottle of water warmed to 40–42° C. After 3–5 minutes, the tail was quickly sponged dry, the end nipped off and *freely flowing* blood collected in the usual manner for white blood counts. With this method, care must be taken in heating the water since slightly higher temperatures will result in blistering of the tail. The time of

[1]Kindly supplied by Hoffmann-LaRoche, Inc.

immersion must be sufficiently long to insure good circulation in the tail.

Using this technique, we were able to obtain tail blood counts which corresponded reasonably well with those obtained on heart blood at the same time and which ran within reasonable limits of daily variation. Therefore, this technique utilizing tail blood was used in most cases rather than that requiring cardiac puncture which often results in fatalities. The total number of neutrophiles (absolute neutrophile value) computed from these figures are, therefore, considered reliable.

All animals were weighed daily. Water and fox chow were available to all animals at all times.

Results

Tables I and II indicate that with a dosage of Presidon, which was sufficiently high to keep the animals in a drowsy

TABLE I

Effects of injections of 200 gamma Presidon/gram body weight/day on mice for a 30 day period

	Number of Mice in Group	Mean Initial Weight of Grp. (grams)	Mean Final Weight of Grp. (grams)	Mean Gain or Loss per Group (grams)		Differences between Mean Gains or Losses (grams)	t	P
Group I								
185 days old								
Subcutaneous	4	28.5	26.5	–2.0				
					>	4.5	6.97	<.01
Control	4	28.4	30.4	2.0				
					>	4.62	11.33	<.01
Intraperitoneal	4	27.75	25.1	–2.62				
Group II								
135 days old								
Subcutaneous	3	27.67	24.67	–3.0				
					>	5.8	6.96	<.01
Control	3	28.2	31.0	2.8				
					>	6.3	5.98	<.01
Intraperitoneal	3	28.0	24.5	–3.5				
Group III								
90 days old								
Subcutaneous	3	24.8	23.2	–1.70				
					>	5.45	16.5	<.01
Control	4	25.25	29.0	3.75				
					>	4.55	20.57	<.01
Intraperitoneal	3	26.0	25.2	–0.80				
Group IV								
50 days old								
Subcutaneous	4	20.0	20.75	0.75				
					>	3.25	5.14	<.01
Control	6	19.0	23.0	4.0				
					>	2.9	3.83	<.01
Intraperitoneal	5	20.4	21.5	1.1				

Note:
"t", determined by mathematical analysis of variants, indicates the relative significance of the weight differences obtained in the study.
"P" is the probability that a difference as great or greater than the one observed would arise by chance through random sampling.

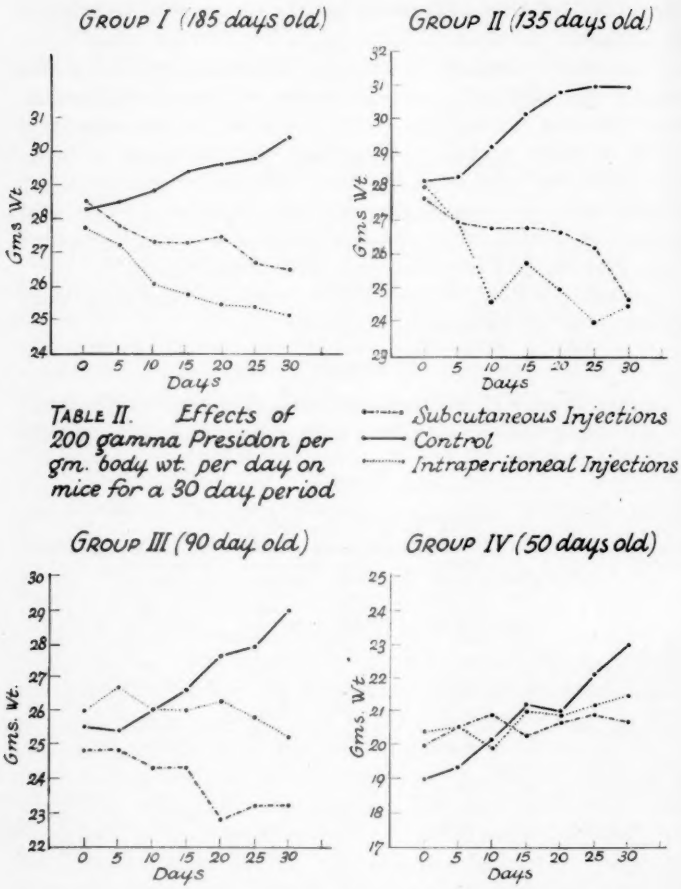

TABLE II. Effects of 200 gamma Presidon per gm. body wt. per day on mice for a 30 day period

state, there was an inhibition of growth (weight gain). Older animals appeared not to gain weight while the younger animals did not appear to show skeletal growth.

Presidon was not found to produce agranulocytosis under the conditions of this experiment. Some changes, however, were found which did indicate the possibility of deleterious effects (Table III).

TABLE III

The Effect of Presidon on the blood of cells of mice

Exper. Animal		BEFORE PRESIDON WBC/mm³ (Counts on 4 successive days)	Avg. of 4 WBC cts.	Diff. (Avg. of 4 bleedings) Eos. %	Bas. %	Non Seg. %	Seg. %	Lym. %	Mon. %	Avg. of 2 WBC /mm³	AFTER PRESIDON Diff. (Avg. of 2 bleedings) Eos. %	Bas. %	Non Seg. %	Seg. %	Lym. %	Mon. %	Abs. No. of Neutro. Bef. Pres.	Aft. Pres.
Group I—185 days age—Presidon administered subcutaneously																		
5♀	Tail Blood	9000– 9700– 8100– 9000	8950	0	0	5	33	60	2	6700	0	0	4	31	64	1	3401	2345
6♀	Tail Blood	8500– 8200– 9000– 8700	8600	1	0	4	36	59	0	6400	1	0	5	22	71	1	3440	1728*
8♀	Tail Blood	6700– 7000– 7500– 7800	7250	0	0	3	37	59	1	7300	2	0	6	30	59	3	2900	2628
9♂	Tail Blood	9400– 8900– 9000– 9600	9225	1	0	8	40	50	1	8900	1	0	2	38	58	1	4428	3560
Group I—185 days age—Presidon administered intraperitoneally																		
11♂	Heart Blood	8050– 9400– 9000– 8800	8813	2	0	5	31	62	0									
	Tail Blood	8100– 9600– 9800– 9600	9025	0	0	2	26.5	69.5	2	6500	0	0	3	26	68	3	2572	1865
12♂	Heart Blood	5250– 5650– 5100– 5850	5463	0	0	3	29	64	4									
	Tail Blood	6200– 6000– 5600– 6100	5975	0	0	4	28	65	3	7950	1	0	6	21	70	2	1912	2146
13♀	Tail Blood	5800– 5100– 5000– 5650	5388	1	0	3	39	56	1	4700	0	0	1	30	69	0	2263	1457
17♀	Tail Blood	6000– 5900– 5100– 5800	5700	0	0	5	31	62	3	3400	0	0	4	26	70	0	2052	1020
Group II—135 days age—Presidon administered subcutaneously																		
26♂	Heart Blood	8500– 8650– 8950– 8000	8525	1	0	6	38	55	0									
	Tail Blood	7950– 9650– 8000– 8750	8575	2	0	3	39	54	2	7200	0	0	3	30	67	0	3602	2376
29♀	Tail Blood	10000– 9800– 8900– 9000	9425	0	0	2	31	66	1	9950	1	0	4	34	60	1	3110	3781
31♂	Heart Blood	6850– 6600– 7150– 6900	6875	2	0	4	40	53	1									
	Tail Blood	6100– 7300– 7450– 7000	6963	1	0	3	37.5	57	1.5	12000	0	0	2	11	87	0	2820	1560
Group II—135 days age—Presidon administered intraperitoneally																		
34♂	Heart Blood	7900– 9600– 8100– 7550	8287	1	1	7	31	59	1									
	Tail Blood	8200– 8100– 9050– 8200	8387	0	0	6	33	59	2	8800	1	0	4	36	57	2	3271	3520
35♀	Tail Blood	10000–10600–11000– 9800	10350	0	0	3	25	71	1	5600	0	0	6	24	68	2	2898	1680
36♀	Tail Blood	7250– 8000– 8600– 7850	7913	1	0	4	36	57.5	1.5	5900	0	0	2	28	68	2	3165	1770

*Animals showing a drop of 1500 neutrophiles/mm³.

TABLE III—(Continued)

The Effect of Presidon on the blood of cells of mice

Exper. Animal		BEFORE PRESIDON WBC/mm³ (Counts on 4 successive days)	Avg. of 4 WBC cts.	Diff. (Avg. of 4 bleedings) Eos. %	Bas. %	Seg. %	Non Seg. %	Lym. %	Mon. %	Avg. of 2 WBC /mm³	AFTER PRESIDON Diff. (Avg. of 2 bleedings) Eos. %	Bas. %	Seg. %	Non Seg. %	Lym. %	Mon. %	Abs. No. of Neutro. Bef. Pres.	Aft. Pres.
Group III—90 days age—Presidon administered subcutaneously																		
43 ♂	Heart Blood	5950– 5600– 5950– 6000	5875	0	0	3	42	55	0									
	Tail Blood	5750– 7700– 6300– 6550	6575	3	0	3	24	69	1	10350	0	0	0	10	89	1	1775	1035
45 ♂	Heart Blood	5900– 5950– 5050– 5650	5625	2	0	4	34	59	1									
	Tail Blood	5800– 6800– 5050– 5200	5712	1	0	2	32.5	62.5	2	8850	0	0	3	24	72	1	1971	2389
51 ♂	Heart Blood	7000– 6850– 6300– 7000	6790	0	0	5	38	56	1									
	Tail Blood	8400–10000– 9400– 8200	9000	2	0	5	42	50	1	6200	2	0	0.5	21.5	75	1	4230	1364*
Group III—90 days age—Presidon administered intraperitoneally																		
56 ♂	Tail Blood	10500–10100– 9300–10000	9975	2	0	7	34	56	1	4700	1	0	0	32	66	1	4090	1504*
57 ♀	Tail Blood	10700–11200–11400–11900	11300	0	0	2	24	73	1	5950	0	0	0	21	78	1	2938	1250*
58 ♀	Tail Blood	9000– 8700– 8850– 9000	8887	3	0	6	25	65	1	5100	0	0	4	21	73	2	2755	1275
Group IV—50 days age—Presidon administered subcutaneously																		
158 ♀	Tail Blood	8500– 7200– 8900– 7050	7912	1	0	5	41	51	2	4625	1	0	0	24	75	0	3640	1110*
160 ♀	Tail Blood	7900– 6800– 7750– 7000	7362	0	0	6	38	55	1	7150	1	0	4	41	53	1	3239	3217
161 ♂	Tail Blood	8000– 7000– 8600– 9300	8225	2	0	3	32	63	0	8700	0	0	0	31	69	0	2879	2697
164 ♂	Tail Blood	8150– 9650– 9300– 9800	9225	0	0	6	30	63	1	8250	1	0	2	37	60	0	3321	3217
Group IV—50 days age—Presidon administered intraperitoneally																		
159 ♀	Tail Blood	7750– 7000– 7800– 8100	7662	1	0	5	36	57	1	3100	2	0	0	24	74	0	3141	744*
163 ♂	Heart Blood	5050– 6500– 5800– 5200	5637	0	0	2	39	58	1									
	Tail Blood	6050– 6100– 5900– 6300	6087	1	0	2	45	52	0	3850	0	0	0	24	76	0	2861	924*
166 ♂	Tail Blood	7900– 7550– 8150– 8700	8075	2	0	8.5	35	53	1.5	7900	0	0	0	23	77	0	3513	1817
168 ♀	Heart Blood	9200– 8800– 9000– 8750	8937	0	1	6	48	44	1									
	Tail Blood	9050– 8300– 9650– 8950	8937	1	0	4	40	53	2	5600	0	0	1	46	53	0	3932	2632
169 ♀	Tail Blood	7300– 7700– 7600– 6850	7362	1	0	8	42	48	1	3550	1	0	6	49	44	0	3681	1952*

*Animals showing a drop of more than 1500 neutrophiles/mm3.

In eight animals, there was a drop of 1,500 neutrophiles/ mm.^{3} blood following the treatment period which is more than a normal daily variation.

The bone marrows of these eight mice showed a "left shift." This shift toward immaturity was severe in two animals (#159 and #163). However, none showed bone marrow damage.

While the purpose of this study with the use of a narcotic dose is preliminary in nature, it suggests that the plan might be fruitfully extended with the use of doses somewhere between the narcotic and lethal levels. Some animals should be allowed to recover from the treatment in order to learn whether the circulating neutrophiles would return to pretreatment levels. It appears that the weight of treated animals might be used as a criterion for the deleterious effect of this and other drugs influencing haemopoiesis.

II. *In Vitro* Studies

Preliminary to testing the toxicity of Presidon on human blood cells, it was believed advisable to determine the minimal quantity which would result in total inhibition (MID) of outgrowth from various chick embryo explants and the dosage level at which the first evidence of injury could be detected (LID). Not only would the data obtained with these methods make possible comparisons between the toxicity of Presidon and a series of other clinically useful drugs, but would also provide information as to whether splenic tissue would prove to be more sensitive than explants of muscular or nervous tissue. Details of the techniques for evaluating toxicity with tissue cultures as used in this laboratory have been described in papers by Painter, Pomerat, and Ezell ('49); Pomerat ('51).

Materials and Methods

Tissue culture medium consisted of equal parts of heparinized cockerel plasma and tissue extract prepared from 7 day incubated chick embryos. These materials were delivered onto 7/8 inch square #1 sterile cover glasses from 27 gauge needles attached to 1.0 ml. tuberculin syringes. Chick embryo explants cut approximately 1 mm. square were derived from 7–9 day hearts, 9 day spinal cords and 18 day spleens.

Presidon was incorporated at various concentrations in equal volumes with the embryonic extract. In preliminary tests, the drug was made up from a stock solution containing 10 mg./ml. so as to yield a final dilution within the clotted medium of 1:4, 1:40, 1:400, 1:4000 and 1:40,000. Four hanging-drop preparations were used for each concentration and for each tissue. On the basis of the outgrowth obtained, subsequent experiments were designed so that the medium contained concentrations of Presidon intermediate between those producing total inhibition and those in which the quantity and appearance of the cells resembled control cultures.

Results

Table IV summarizes the results obtained on more than 200 hanging-drop cultures used to determine the effect of

TABLE IV

Outgrowth from chick embryo explants in relation to the amount of Presidon included in the clotted medium

MID—Minimal dose for total inhibition
LID—Lowest concentration showing evidence of injury to cells

	Presidon—millimoles per ml.	
	MID	LID
Heart (7–9 day).....	30.0 – 22.5	9.0 – 4.5
Cord (9 day).....	30.0 – 22.5	2.25 – 1.12
Spleen (18 day).....	30.0 – 22.5	4.5 – 2.25

Presidon upon the outgrowth of cells from chick embryonic explants. No difference could be found in the quantity required to produce total inhibition of outgrowth (MID) between heart, spleen and spinal cord explants. No outgrowth was obtained in the presence of 30 millimoles of Presidon per ml. of medium while some evidence of cellular activity was seen in cultures containing 22.5 millimoles per ml. Cells from spleen explants appeared to be somewhat more sensitive to the drug than fiber outgrowth from spinal cord, while fibrocytes from heart fragments showed the highest resistance to Presidon. Experience with tissue culture assay technique has regularly shown that splenic explants are particularly

sensitive to deleterious agents. It is not believed that the difference in the LID found for Presidon could be used as a reliable index of its potential capacity to damage blood cells within the short-term period of cultivation used in our tests.

According to Koppanyi, Herwick, Linegar, and Foster ('40), the LD_{50} for Presidon is 350 mg./kg. when injected intravenously in rabbits. This may be expressed by the ratio 1:2857. The LD_{50} was found to be 1062 mg./kg., or 1:942, for Presidon injected subcutaneously in mice. Total inhibition of cellular outgrowth from tissue cultures of chick embryo heart, spinal cord and spleen explants was obtained with the use of 5 mg. per ml., while slight outgrowth was obtained in the presence of 3.75 mg. per ml. These values represent ratios of 1:200 and 1:267, respectively. The inhibitory dose *in vitro*, therefore, was approximately ten times greater than the rabbit intravenous dose and four times greater than the lethal subcutaneous dose for the mouse.

Comparisons of *in vitro* and *in vivo* toxicity doses have always indicated higher values for total inhibitory effects upon cells in culture medium studied with our methods. Thus the *in vitro* dose for total cellular inhibition with paraldehyde was found to be twice greater, strychnine ten times greater, caffeine 40 times greater, while metrazol required a quantity nearly 2,000 times greater for lethal effects than was required for experimental animals. These observations have demonstrated that fatal doses *in vivo* probably affect particular cell systems involving crucial physiological mechanisms while other cell species retain a relatively high resistance to the toxic agent (*cf.* Painter, Pomerat, and Ezell, '49).

Summary and Conclusions

A total of 46 mice in four age groups (185, 135, 90 and 50 days) were given 200 gamma of Presidon per gram of body weight for 30 days by both subcutaneous and intravenous routes. Older animals did not gain weight while younger animals did not appear to show skeletal growth.

Agranulocytosis was not produced under these conditions, although a marked reduction in the absolute number of neutrophiles was observed in several animals. The bone marrow

of these animals showed a "left shift" which was severe in two animals but showed no actual bone marrow damage.

The toxicity of Presidon was also tested with cultures of chick embryonic tissues. The minimum quantity of the drug required to inhibit all outgrowth from explants of heart, cord and spleen was 30 millimoles per ml. (MID) while some cellular activity was seen in the presence of 22.5 millimoles per ml. Expressed in millimoles per ml., the least amount of the drug showing slight injury as compared with untreated control cultures (LID) was 9.0 for heart, 4.5 for spleen and 2.25 for the spinal cord.

It is believed that inhibition of growth, particularly of the skeleton of young animals, might be useful in evaluating the toxicity of drugs suspected of damaging haemopoietic tissue. Animals showing a significant drop in absolute neutrophile count should be allowed to recover in order to learn whether circulating neutrophiles would return to pretreatment levels.

While the spleen in culture did not seem significantly more sensitive to the action of Presidon than heart and spinal cord explants, further studies are indicated. Continued efforts should be made to establish a technique for evaluating haemopoiesis *in vitro*. In addition to lymphoid tissue and the bone marrow, efforts should be made to improve methods for cultivating the buffy coat from human subjects.

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SOME CARDIOVASCULAR ACTIONS OF DINITROPHENYLMORPHINE HYDROCHLORIDE*

R. A. Huggins and A. R. Bryan

The compound 2,4-dinitrophenylmorphine (DNPM) is of interest because it combines a metabolic and central nervous system stimulant with an analgesic central nervous system depressant. A survey of a number of the pharmacological properties of the compound has been made by Emerson *et al.* (1938) and Abreu *et al.* (1938). The data presented by Emerson *et al.* (1938) relating to the cardiovascular system indicate that DNPM when injected intravenously in rabbits previously anesthetized with 50 mg./kg. of di-allyl barbituric acid in urethane solution causes a prompt decrease in blood pressure with a slow recovery to normal levels. With the drop in blood pressure, cardiac acceleration occurs, but in unanesthetized dogs and rabbits given DNPM a tendency for a slowing of the pulse is observed.

While making a study recently on a comparison of the cardiovascular actions of morphine and its derivatives (Huggins *et al.*, 1951), we were unable to obtain sufficient DNPM to include data on this compound. Since then, enough DNPM was synthetized by the method of Mannich (1935) in the Department of Pharmacology and Toxicology of The University of Texas Medical Branch to enable us to present the following data.

Experimental

Methods.—Dogs were anesthetized with sodium barbital, 300 mg./kg., and chlorazol fast pink, 80 mg./kg., was used as the anticoagulant. Blood pressures were obtained with a Hamilton manometer (Hamilton *et al.*, 1934) and the camera was run at a speed sufficient to give pulse contours which were used to calculate the cardiac output by the method of Hamilton and Remington (1947). Blood flow through one carotid artery was measured with the recording rotameter (Gregg *et al.*,

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1942). Intra-arterial injections were made into the distal end of the rotameter and intravenous injections into a femoral vein.

Results.—Typical cardiovascular responses in the dog to the injection of DNPM are shown in figure 1. Injection of 0.1 mg./kg. intravenously had no effect. The delayed changes are not related to the actions of DNPM but occur occasionally with preparations of this type particularly if there is a rapid heart and high blood pressure. A new base level is usually established at a lower pressure and will be maintained until the dog deteriorates. That such a level was established is seen in figure 1. A period of 20 minutes elapsed between the last point after injection of 0.1 mg./kg. intravenously and the control determinations for the intra-arterial injection. The only difference is a slight increase in heart rate.

When 0.1 mg./kg. of DNPM is administered intra-arterially the first effect noted is a marked increase in flow to the head within 15 seconds and a precipitous drop in vasomotor resistance as indicated by the broken line. The same effect is noted on the hind leg if the rotameter is inserted into a femoral artery. These changes are observed before any marked systemic effect and are indicative of the primary dilating action of DNPM on the blood vessels. The systemic effects are characterized by a precipitous drop in systolic and diastolic pressures, heart rate, vasomotor resistance, and head flow with a variable effect on cardiac output.

DNPM given intravenously in 1 mg./kg. doses produces the same general effect with the difference that the drop in cardiac output and subsequent increase are greater and the decrease in vasomotor resistance is not as marked.

Discussion

On comparing the cardiovascular actions of DNPM with those of morphine several interesting differences appear. The intravenous injection of morphine (1–2 mg./kg.) results in a decrease in blood pressure and an increased heart rate while DNPM causes a drop in blood pressure and in heart rate. The decrease in heart rate with DNPM suggests that it possesses a central vagal stimulating effect absent in morphine. The only other morphine derivative we (Huggins *et al.*, 1951)

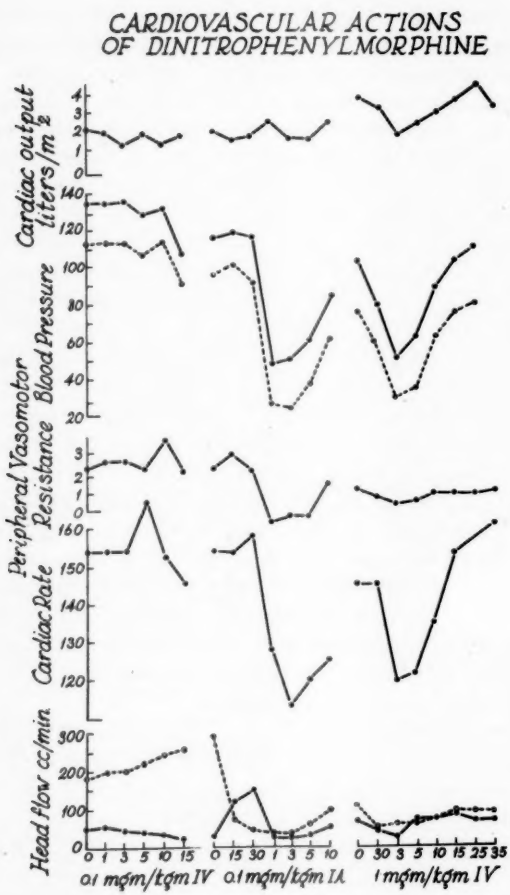

FIG. 1. A graph of the typical cardiovascular events on injection of DNPM. The broken line of the head flow section of the graph shows the trend for head resistance. This was calculated by the formula (MP — 20) / HF min. Vsaomotor resistance was calculated by the formula (MP — 20) / F, where MP is the mean arterial pressure in mm. Hg. and F, the blood flow in cc. / sq. M / sec. The time scale is in minutes except for the intraarterial injection where 15 and 30 are seconds and the 1 mg. / kg. intravenous injection where the first 30 also represents seconds.

have found to have this action is metopon. With the slowing of the heart rate some decrease in cardiac output might be expected but not a decrease in stroke volume. That a decrease in stroke volume does occur may result from the drop in vasomotor resistance with pooling of blood in the periphery, thus restricting venous return. Morphine, however, produces an equivalent decrease in vasomotor resistance but venous return is apparently not impeded as the minute output of the heart increases with the heart rate.

A comparison of the cardiovascular effects of DNPM with morphine has been made but because the phenolic hydroxyl group in DNPM is "muzzled" it might be more valid to consider its relationship to codeine. Codeine, however, has the same cardiovascular actions as does morphine with the exception that the depressor action of codeine, as the dose is increased, is very much longer than that observed with morphine.

There are in addition to the differences mentioned important points of similarity. In common with morphine and codeine, DNPM has a primary dilating effect on blood vessels as is shown by increased flow through a vascular bed if DNPM is administered intra-arterially. This direct effect on blood vessels could contribute to the decreased vasomotor resistance that always occurs but it is doubtful that it is the main factor. A central effect on the vasomotor center is suggested by the marked accentuation of cardiovascular actions when injection is made into a carotid artery. A dose, in the case of DNPM, of 0.1 mg./kg. when given intravenously had no cardiovascular action but the same amount injected into the carotid artery produced a response not quite equaled by 1 mg./kg. intravenously. The same directional responses occur with morphine and codeine but Schmidt and Livingston (1933) considered the depressor actions of intravenous morphine on blood pressure to be largely peripheral. Our data suggest that at least in the dog morphine and a number of derivatives including DNPM have either a direct action on the vasomotor center, or cause an imbalance of impulses impinging on this center, producing depression of vasomotor resistance and a fall in blood pressure. The direct action of morphine or DNPM on blood vessels is then secondary in contributing to the decrease in vasomotor resistance.

SUMMARY

(1) 2,4-Dinitrophenylmorphine injected intravenously or intra-arterially in dogs causes a decrease in vasomotor resistance, blood pressure and heart rate. The effect on cardiac output is variable.

(2) The variable effect on cardiac output is probably the result of pooling of blood in the periphery which results in decreased venous return.

(3) Dinitrophenylmorphine, like morphine and codeine, has a central action causing a decrease in vasomotor resistance. It is probable that the direct dilating action of these compounds on blood vessels is of secondary importance in the decreased vasomotor resistance.

(4) Dinitrophenylmorphine, in contradistinction to morphine or codeine, causes bradycardia.

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RATIO OF BONE WEIGHT TO LIVE BODY WEIGHT OF THE WISTAR RAT*

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Introduction

In determining the skeletal uptake of radio-isotopes in Wistar strain rats, a value of the percentage of bone in the rat body and rat carcass was desired. Values of approximately 6% bone in the body weight of the rat have been reported (1) on a small series of rats, but because of possible differences in age, sex, diet, etc., it was decided to repeat these studies. The following determination of the ratio of bone weight to body and carcass weight was performed, using animals from the same age group, and under the same conditions as were used in the isotope distribution studies. The body after the pelt and viscera had been carefully removed was termed the carcass.

Procedure

Ten young adult albino male rats of the Wistar strain weighing from 236 to 286 g., and 4½ months of age were selected. The rats were anaesthetized with nembutal, and vivi-perfused. The bones of the left rear leg, left fore-leg, and shoulder girdle (including the clavicle and scapula), left half of the mandible, the calvarium and the sternum were dissected out and meticulously cleaned of muscle and connective tissue. These bones were weighed and then heated at 45° C. in 10% KOH for 4 hours. The remainder of the skeleton was heated in a similar manner in a separate beaker. The sample bones and remainder of the skeleton were weighed after drying in air, and after drying overnight in an electric oven at 45° C. From this data the "wet" weight of the total skeleton was computed, correcting for loss of bone weight which occurred upon heating

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with KOH. The percentage bone in carcass and percentage bone in the animal (live weight) were calculated. All bones were then baked for 2 hours at 120° C., reweighed, and the above values and percentages were computed using the "baked" bone data. These studies were repeated on 5 young rats, 6 weeks of age, and on 5 older rats, 8 months of age, to determine whether age will seriously affect the values obtained for the "young adults."

As shown in Table 1, bone weight expressed in terms of percent carcass weight shows a greater standard deviation and

TABLE 1

Ratio of Bone Weight to Live Weight and Carcass Weight

Rat No.	Age	Live Wt.	Carcass Wt.	Skeletal Wt. Wet	Skeletal Wt. Baked	% Bone in Carcass Wet	% Bone in Carcass Baked	% Bone in Animal Wet	% Bone in Animal Baked
15	6 wk.	130	65.5	11.52	11.0	17.0	16.8	8.6	8.46
16		136	61.5	9.4	9.7	15.3	15.7	6.9	7.0
17		124	61.5	8.3	9.35	13.5	15.3	6.7	7.5
18		139	74	8.55	8.6	11.6	9.3	5.0	4.95
21		156	87	11.7	11.57	13.5	13.3	7.55	7.44
Mean		137	69.9	9.82	10.05	14.18	14.08	6.97	7.07
				Standard Deviation—		2.05	2.57	1.36	1.67
				Coefficient of Variation—		14.45%	18.3 %	19.5 %	23.6 %
5	18 wk.	247	141.9	27.7	26.0	19.5	18.3	11.5	10.05
6		286	153.5	18.4	19.25	12.0	12.05	6.42	6.74
7		242	131	17.2	18.8	13.1	14.35	7.0	7.77
8		244	134	19.6	19.6	14.6	14.6	8.05	8.05
9		259	114	19.7	17.9	17.3	15.6	7.6	6.90
0		296	164	20.1	19.75	12.3	12.05	6.8	6.68
1		290	171	22.0	22.13	12.85	13.0	7.6	7.7
2		238	134.5	15.5	15.6	11.5	11.6	6.52	6.55
3		253	143.5	18.7	18.75	13.0	13.05	7.4	7.4
4		242	143	15.3	16.2	10.6	11.3	6.33	6.7
Mean		259.7	143	19.42	19.39	13.67	11.76	7.08	7.16
				Standard Deviation—		2.07	2.07	0.61	0.568
				Coefficient of Variation—		15.15%	17.60%	8.62%	7.94 %
19	32 wk.	270	163	20.9	20.5	12.8	12.5	7.75	7.6
20		322	190	21.05	21.3	11.1	11.2	6.63	6.6
22		320	204	20.39	22.2	10.0	10.9	6.36	6.95
23		381	230	19.2	21.0	8.35	9.15	5.04	5.5
24		406	242	20.0	22.3	8.3	9.2	4.91	5.5
Mean		333.3	205.8	20.31	21.4	10.13	10.59	6.11	6.43
				Standard Deviation—		2.4	1.42	1.22	0.92
				Coefficient of Variation—		23.6 %	13.4 %	20.0 %	14.3 %

coefficient of variation than does bone weight percentage of the live body weight. Consequently the values of bone weight percentage of the live animal weight are considered preferable. The figures using baked bone weight vary but little from the wet bone weight and although the coefficient of variation for the baked bone weight in the animal of the first group is slightly lower than that for the wet bone weight, the slight variation does not appear to justify the extra time and expense of baking the bones. The values of animal number 5 were omitted from the determinations as it was several standard deviations removed from the mean figure.

Summary

The best value for computing the skeletal weight of rats that weigh about 250–290 g. is the live body weight multiplied by .0708. This varies slightly from the value of 6% given by Donaldson in his monograph on "The Rat." Similar values for the young and the old rat groups also fall around 6 to 7% but exhibit greater variations.

REFERENCE

1. Donaldson, H. H., No. 6, American Memoir Series, Wistar Series, Wistar Institute, 1925.

Animal
Baked
8.46
7.0
7.5
4.95
7.44
7.07
1.67
23.6 %
10.05
6.74
7.77
8.05
6.90
6.68
7.7
6.55
7.4
6.7
7.16
0.568
7.94 %
7.6
6.6
6.95
5.5
5.5
6.43
0.92
14.3 %

A PHARMACOLOGIC ANALYSIS OF DRUGS USED IN ALLERGY

CHAUNCEY D. LEAKE*

Allergy is an increasingly important problem. Its symptomatology is complex, its etiology complicated and obscure, and a confusing variety of drugs is recommended for its management. In order to use these drugs effectively, it is wise to keep in mind their demonstrable biological actions in relation to significant factors involved in allergy.

Symptomatology

Allergic manifestations may be classified in three ways: (1) those localized in tissues and organs, common examples of which are hay fever and asthma; (2) generalized acute and severe symptoms, as in anaphylactic shock, and (3) generalized mild and chronic reactions, of which fever from an infection may be considered typical.

The first satisfactorily clear descriptions of symptoms corresponding to what is now called allergy were made by London clinicians. John Bostock in 1819 described "summer catarrh," now known as hay fever. In 1831, John Elliotson, of hypnotism fame, recognized pollen as the cause of hay fever. Henry Salter in 1860 gave the first satisfactory clinical account of asthma. Sick headache was realized to be related to hay fever by Liveing in 1873. However, French workers laid the basis for modern concepts of allergy. Anaphylactic shock was described and named by Portier and Richet in 1902, while Arthus reported the local inflammatory tissue pathology in 1903. Pirquet observed serum sickness in 1905. In 1907 he coined the

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*Vice-President, The University of Texas, for the Medical Branch, Galveston. This sketchy review was prepared primarily for the benefit of general practitioners; specialists in allegery, however, may find provocative stimulus in some of the ideas suggested in it. It was presented at the General Scientific Meeting of the 99th Annual Session of the American Medical Association, San Francisco, June 27, 1950, and at the St. Louis meeting of the Southern Medical Association, November 16, 1950. Thanks to the officials of these organizations, arrangements have been made for publication here. Thanks are also gratefully extended to Dr. Ralph Bowen of Houston and to Dr. Edmund L. Keeney of San Diego for helpful advice and criticism.

term, "allergy." Food allergy was noted by the American pediatrician, Schloss, in 1912, and Landsteiner analyzed drug allergy in 1914. In 1921 Barbour implied that fever might be considered a generalized mild form of allergic reaction.

Pathologic Physiology of Allergy

Barbour showed that contact with provocative proteins and certain toxic agents causes water imbibition by cells, with congestion where the imbibition occurs and resulting hemoconcentration if the process is relatively general. The localized congestion and swelling causes many of the local symptoms of allergy. If hemoconcentration occurs, there may not be water enough available for loss by evaporation from lungs and skin. With this reduction in the cooling mechanism, body temperature rises, and fever symptoms are noted.

Meanwhile in 1910 a classic pharmacologic study had been made by Dale and Laidlow of an extraordinary component of living cells, histamine. This chemical was shown by Lewis and Grant to give a triple response when injected into the skin: vaso-dilation at the point of injection, an axon-reflex flare, and tissue swelling, or a wheal. Dale, whose genius has revealed so much of the interrelationships of the powerfully acting chemical regulators of body function, adrenalin and acetylcholine, suggested that histamine is released from injured cells in allergy. Dragstedt and Gebauer furnished proof of this theory and it was concluded that allergic symptoms result from the release of histamine, heparin, and possibly choline from sensitized cells, due to changes in permeability resulting from the antigen-antibody reaction. How this change occurs is not yet clear.

Meanwhile, Wolbach had demonstrated how ascorbic acid is essential for binding cells tightly together. Others later showed the importance of carotenoids, calcium, and hyaluronic acid in maintaining intercellular cement and cell membrane stability (Reid). Recently ACTH and adrenal-cortex steroids have also been implicated in the maintenance of healthy cellular colloidal stability (Chase, White, and Dougherty). Currently, tissue culture studies of human nasal mucosa reveal the effects of antigens, to which the mucosa is sensitive,

in breaking the lipo-protein granules of the cells, in indicating gelation of cytoplasm, and in slowing mitachondrial and ciliary movement (Rose, Pomerat, and Danes). The tendency to gel under the influence of the antigen suggests the water imbibition and swelling implied by Barbour.

Etiologic Factors in Allergy

These many considerations reflect the multiplicity of factors in the complex physico-chemical reactions involved in allergy. There are, first, the inherited complex of metabolic and enzyme patterns of cells and cellular membranes. What physico-chemical details are concerned in the deviations of cells in allergic symptoms can merely be guessed. But an inherited tendency seems to be present.

There is, second, presumptive and indirect evidence that vitamin deficiencies, especially of carotenoids, ascorbic acid, calciferols and tocopherols, open the way for allergic responses of cells. The carotenoids and ascorbic acid are necessary for firm binding of cells together. Relative lack results in loosening of intercellular cement, with better chance for provocative allergens to come in contact with cell surfaces. The calciferols and tocopherols seem to be needed for healthy cell membranes, the former in relation to calcium, and the latter in relation to obscure physico-chemical systems. Similarly, ACTH and adreno-cortical steroids seem to be essential for maintaining optimum sodium-potassium balances within cells and cell membranes, and in preserving the integrity of enzyme systems necessary for the maintenance of cellular stability. Relative lack of the vitamins or hormones concerned may make the cell more vulnerable to injury by contact with provocative allergens, or may make possible the more extensive immediate release of histamine than ordinarily is the case in healthy cells. It may also be possible that, when cells are not in healthy balance, the histamine release cannot be handled in the ordinary way to prevent untoward reactions.

The immediate allergic reaction results from the interaction of a protein molecule comprising the allergen, with some particular molecule of the cell surface, in such a way as to disturb

the cell surface, release histamine, and induce water imbibition by the cell. This reaction occurs only to certain proteins to which the cell surface has become "sensitized." There is merely speculation regarding the phenomena of sensitization. We simply do not yet know enough about the physico-chemical factors involved in the complex patterns of molecules comprising cell membranes and cells to form a satisfactory theory involving sensitization. There is no point in fooling ourselves into thinking we have explained sensitization when we use a terminology from which *a priori* conclusions have already been drawn.

Certain it is, however, that the allergic reaction results in histamine release. Histamine forms loose combinations with protein, and indeed such combinations have been used (with little success) to reduce sensitization or to relieve allergy (Sheldon, Fell, Johnston, and Howes). The synthesis and hydrolysis of histamine is regulated, in part, by an enzyme called by Best, its discoverer, "histaminase." This enzyme presumably has been isolated, and preparations of it have also been used to treat allergic conditions. The results, however, have not been promising (Rose).

The biological effects of histamine are well known and thoroughly reproducible (Selle). It is presumed that histamine comes from histidine, or from unstable protein combinations. Its inhibition under ordinary circumstances seems to be accomplished by the balanced enzyme systems involving histaminase, and combinations with proteins. In allergy, however, these systems do not operate in the usual manner, and enough histamine accumulates to exert its characteristic pharmacologic or toxic action. If this action can be blocked by competitive compounds, the effects do not appear. This apparently is what happens in the case of the antihistamine drugs. This may also happen in the case of desensitization, when immune bodies may be formed from globulins in the reticulo-endothelial cells, which may block allergens from reacting with cell surfaces (Burnet).

Allergy results from the contact or ingestion of foreign colloid complexes to which sensitization of the cells of the

body has occurred. These strange allergens may come from dust, pollens, foods, clothing, or various plant or animal materials in the environment of the sensitive person. An obvious method of handling allergy is to try to find the particular protein to which the individual is sensitive, and then to prevent contact with it. Removal of the allergic patient to a pollen-free place is a common method of handling hay fever. Elimination diets, as developed so successfully by Rowe, are effective in the management of food allergies. In order to protect a sensitized individual from contact with the protein to which he is allergic, it is essential to be certain of the particular protein involved. This necessitates careful diagnostic procedures using standardized procedures and reliable antigens. These diagnostic procedures are not without anaphylactic danger (Harris and Shure).

Among other etiological factors which may be involved in allergy one may consider unstable antibody formation, or abnormal globulin synthesis or metabolism as discussed by Burnet. These may reflect disturbances of metabolic and enzyme patterns of the body cells in complementariness of immune-body synthesis with reference to antigen or allergen. Sensitization may follow stripping of protein molecules from cell surfaces, so that antibody formation is prevented.

It appears that allergic tendencies may be reduced by an adequate protein intake and by the maintenance of a satisfactory balance of vitamins and steroid hormones. Accurate diagnosis may reveal the specific protein to which the allergic individual is sensitized so that the individual may be protected against contact with this specific allergen. Psychogenic factors, especially those operating in anxiety or emotional stress, may be involved in the allergic patient. These nervous factors may be concerned in various phases of the "alarm reaction" (Selye), and by interference with adrenocortical hormones, predispose toward allergic reactions when contact occurs with provocative toxins. After attention to the control of predisposing factors in regard to these details, the management of allergy appears to be a matter of treating the various symptoms which may develop.

Drugs Used in Allergy

The drugs used in allergy may be classified as follows: (1) general cellular stabilizers; (2) chemotherapeutic agents; (3) antipyretic analgesics; (4) smooth muscle relaxants of the purine type; (5) smooth muscle relaxants of the benzylquinoline kind; (6) smooth muscle relaxants of the sympathomimetic variety including local anesthetics; (7) smooth muscle relaxants of the parasympatholytic sort; (8) antihistaminics, and (9) specific and nonspecific protein sensitizers. This is an extraordinarily wide variety of drugs, and indicates clearly the complexity of the modifications in the allergic conditions which may be brought about by the various kinds of chemicals.

Physicians would be wise to remain traditionally skeptical over enthusiastic claims for new exclusive methods for treating allergy, many of which are phrased in flattering but confusing scientific terminology to trap the credulous. Recent examples have included an organic chemical in a concentration of 10^{-15} in conductivity water (about one molecule per cc.) for intramuscular injection (resulting hemolysis from the distilled water giving the reaction), and "aran," a hypothetical substance in air supposed to confer that cheerful buoyant feeling of a crisp fall day.

General Cellular Stabilizers

The general cellular stabilizers may aid in preventing allergy. They include an adequate protein intake for proper globulin synthesis, so that immune bodies and antibodies to various allergens may be formed in a healthy way (Burnet). Healthy cellular stabilization for the prevention of allergy may also be obtained by systematic attention to a healthy vitamin balance and intake, and also to an adequate supply of minerals.

Some success in preventing allergy may also be obtained by detailed attention to the hormonal balance, and to the administration of ACTH or adreno-cortical steroids if indicated. These important agents are effective in preventing and treating symptoms of stress, as so well demonstrated by Selye. There is accumulating evidence that the allergic reaction may be considered to be a manifestation of cellular stress. Bile steroids

may also be found to clear allergy, since allergic symptoms are reported to clear in jaundice (Venulet and Kadlubowski). Thyroid hormones may also be helpful. These are essential for the maintenance of proper oxidative metabolism in cells with respect to energy production.

Allergic conditions can often be effectively treated by the administration of potassium iodide. While this has been on an empirical basis for over a century, it would appear that the compound acts by virtue of its effects on colloid systems, since it stands so high in the lyophillic series. The empirical success of solutions of potassium arsenite in allergy may be due to similar factors. Certainly in the enthusiasm for new methods for treating allergy, the effectiveness of potassium iodide in relieving congestive symptoms should not be forgotten (Feinberg).

Chemotherapeutic Agents

The effectiveness of chemotherapeutic agents in allergy may be secondary to the removal by the chemotherapeutic agent of invading microorganisms producing sub-clinical or mild infections. Allergic symptoms in the upper respiratory tract frequently clear on successful treatment of infections of these areas. The antibiotics have largely supplanted other chemotherapeutic agents in treating infections. If one is tempted to return to the use of organometallic compounds or sulfa drugs, one should remember that there is the possibility that these agents themselves may produce allergic reactions, as indicated from the studies of Landsteiner. The probability of an allergic response to these drugs is considerable. It seems to be around 5% of all individuals to whom these kinds of drugs are administered.

The antibiotics themselves may also produce serious allergic reactions, as so many people have learned to their sorrow in connection with penicillin. The allergic reactions to penicillin have been discussed by Barker. While streptomycin has been demonstrated to have some antihistaminic action, considerable allergic effect appears to occur. This is discussed in Molitor's comprehensive review of the antibiotics. Aureomycin is best given orally to avoid untoward reactions which are common

with parenteral administration. Allergic responses to oral administration, however, have been noted (Parets). Chloramphenicol is relatively free from undesired effect except on intramuscular injection. The polymixins and bacitracin have nephrotoxic properties.

Antipyretic Analgesics

The antipyretic analgesics include the salicylates, aspirin, the cincophens, the aminopyrins, the acetanilids, and a large number of related compounds. All of these agents act according to Barbour's theory by reversing cellular imbibition of water in allergic processes. The net effect of this action is to relieve congestive pain and associated malaise. The release of water from the cells under the influence of the antipyretic analgesics results in hemodilution, with resulting loss of water from the lungs and skins by evaporation, so that the cooling effect reduces the fever that may be involved. Barbour's theory is essentially one for the mechanism of fever, but with respect to the antipyretic analygesics, it may be extended, as has been done, to explain the mechanism of their action.

The antipyretic analgesics are excreted in the urine in four to six hours, often in conjunction with amino acids. The relative rapidity of their excretion requires repeated administration every four to six hours, if effectiveness is to be maintained in the presence of a provocative allergen. Their conjugation with amino acids offers, on Landsteiner's theory, the probability of allergic reaction to these drugs themselves. This is often manifested in the case of aspirin with nasal and upper respiratory congestion, or with urticaria. With the aminopyrines there seems to be a considerable danger from agranulocytosis, which may result from an allergic reaction in bone marrow.

The success of the salicylates and the cincophens in the treatment of rheumatic conditions lend support to the idea that these diseases have an allergic basis. The effectiveness of ACTH and cortisone may also be explained with reference to the same general theory. A direct antihistamine action of sodium salicylate has been described by Swyer.

Smooth Muscle Relaxants

If the allergic reaction involves smooth muscle cells, with characteristic swelling of these cells from water imbibition, spasm may result. This may be a factor in the asthmatic reaction in lung, associated with tissue swelling. It may also be involved in intestinal colic or spasm of the smooth muscle of the urinary tract. Many types of smooth muscle relaxing agents may assist in relieving smooth muscle spasm in allergy. These compounds, however, are not particularly effective in reducing the swelling of the tissue, since they apparently have little influence on the disturbed colloidal situation. Hence, with the exception of the phenyl-alkylamines, they are not ordinarily useful in relieving mucosal congestion or in relieving skin urticaria or other manifestations of skin allergy.

Many purine derivatives are effective smooth muscle relaxant agents. Among them aminophylline is preferred in allergic conditions. It is a mild relaxant, but effectively gives relief from smooth muscle spasm in allergic situations. Of the opium alkaloids, papaverine may also be helpful in the same way.

Aminophylline may also reduce antigen-induced skin wheals, and thus give some relief in chronic urticaria. Since aminophylline has no inhibiting effect on histamine, it would seem that histamine is not solely responsible for all symptoms of allergy.

The purine derivatives are sometimes combined with antihistaminics, as in "Hydryllin" (Searle), or with vasoconstrictor agents such as ephedrine, benzedrine, or neosynephrin. These vasoconstrictor agents also assist in relaxing smooth muscle spasm, and are helpful in combination with purine derivatives when smooth muscle spasm occurs with mucous membrane congestion. The inclusion of caffeine, a characteristic purine compound, with analgesic antipyretics in the treatment of upper respiratory tract congestion and the symptoms of the common cold, may be based in part on its effects in promoting some degree of vasodilation and improved blood and lymph drainage.

The benzyl-quinolines are not popular smooth muscle relaxing agents, and are little used for allergy. Papaverine is one

of the most effective of the opium alkaloids for smooth muscle relaxation. It may aid in relieving upper respiratory congestion and asthmatic symptoms. Thomas Dover's buccaneering mixture of powdered opium and ipecac is a frequent combination for use with antipyretic analygesics for treating upper respiratory congestion and symptoms of the common cold, associated with allergic conditions. This has merit in promoting secretion and reducing sensory annoyance from the affected area.

Sympathomimetic Smooth Muscle Relaxants

The sympathomimetic smooth muscle relaxing agents of value in allergy are the phenyl-alkylmanines, such as epinephrine, ephedrine, benzedrine, and neosynephrin, and many of the local anesthetics. While these compounds are primarily useful in allergy in relieving smooth muscle spasm, they also are effective in alleviating severe skin manifestations of allergy. Epinephrine parenterally is particularly effective for prompt action in acute allergic conditions. It may be satisfactorily administered subcutaneously, and relief is usually to be obtained from less than ordinary recommended doses.

Epinephrine and related compounds are powerful vasoconstrictors. They assist in relieving mucous membrane congestion by causing arteriolar constriction, and thus assisting in "shrinking" the tissues involved. On subcutaneous injection, local vasoconstriction is sufficient to prevent more than minimum absorption of epinephrine. The remainder stays in place, and until destroyed by oxidation within eighteen to twenty-four hours, may be further released for absorption by gentle rubbing over the place of injection. This effect is helpful clinically in dealing with acute severe symptoms when it may frequently be impossible for a trained attendant to be constantly on watch.

While epinephrine parenterally is most satisfactory for acute asthmatic or serum sickness symptoms of allergy, ephedrine orally is useful for sustained action. Neosynephrine spray with mild alkaline buffer is popular for nasal congestion. The inhalation of the volatile benzedrine is also helpful in nasal and upper respiratory congestion. All of these drugs have

some effect in promoting central nervous system stimulation. This is most marked with benzedrine.

The chemical constitution of the phenyl-alkylmanines and many local anesthetic agents is similar to histamine and antihistaminic agents, and thus it might be expected that these compounds have some antihistamine action by virtue of competition with histamine for cell receptors. While this has not been investigated in detail, there is some evidence to indicate that this may be a factor in the effectiveness of these compounds in allergic conditions. The intravenous injection of procaine salts in buffered isotonic solution may be beneficial in generalized acute allergy conditions.

Parasympatholytic Smooth Muscle Relaxants

The parasympatholytic smooth muscle relaxing agents are chiefly compounds of the belladonna group. Of these atropine sulphate seems to be most effective either orally or parenterally for prompt action. Appropriate preparations of the crude drug, belladonna, may be effectively given orally for prolonged action. The inhalation of burning stramonium leaves was once popular for treating asthma. Stramonium preparations have also been recommended orally for relief of allergic conditions. Hyoscine or scopolamine is effective in producing some degree of nervous dissociation, but does not appear to be particularly useful in allergic conditions. Like all alkaloids, those of the belladonna group are inactivated within a few hours in the body, so that administration must be repeated every three or four hours in order to maintain the desired effect. The belladonna alkaloids are powerful so that minimal doses are indicated.

The belladonna alkaloids are helpful in asthmatic conditions and in relief of allergic colic or spasm of the smooth muscle of the intestinal and urinary tract. In these conditions, synthetic compounds, such as trasentin, are also useful. The belladonna alkaloids are frequently administered with cerebral depressants, such as barbitals, for optimum effect in allergies involving psychogenic factors associated with anxiety, apprehension and smooth muscle contraction. The barbitals are also administered with the belladonna alkaloids in order

to avoid some of the central nervous system stimulating effects of these alkaloids. It is important to remember that the belladonna alkaloids are of little value in skin allergies. Their chief effect is to paralyze parasympathetic nerve endings, which do not seem to be involved in allergic reactions of the skin.

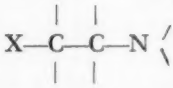
Antihistamine Compounds

The late great French pharmacologist, E. Fourneau (1872–1949), first succeeded in synthesizing sympatholytic compounds which neutralize or reverse the effects of epinephrine. Because of the similarity in chemical configuration of acetylcholine, epinephrine, and histamine, Fourneau's associate, D. Bovet, undertook with his pupil, A. M. Staub, to find compounds which might antagonize histamine, in the same way as compounds have been found to antagonize acetylcholine and epinephrine. One of Fourneau's compounds in 1937 was found to protect guinea pigs against lethal doses of histamine, to antagonize histamine induced smooth muscle spasm, and to protect against anaphylactic shock. The compound was thymoxyethyl-diethylamine (929F). This important observation initiated a vast competitive study of various alkylamino compounds for possible antihistamine effect. Fortunately, many were discovered which have very low toxic or untoward effect, and which are useful clinically as antagonists for histamine, and thus of value in various forms of allergy.

The pharmacologic and clinical evaluation of these many compounds has not been an easy task. Their pharmacologic properties have been reviewed by Loew, and Feinberg has ably summarized the status of their therapeutic efficiency in allergy, with particular attention to the correlation between experimental and clinical observations. The quantitative aspects of the competition between antihistaminics and histamine for cellular receptors has been mathematically investigated by Wells, who has established quantitative values for the competition index of certain antihistaminics in comparison with histamine.

There are several classes of antihistamine compounds: (1) derivatives of ethanolamine, of which benadryl is typical;

(2) relatives of ethylenediamine, of which neoantergan, pyribenzamine, thenylene, and chlorothen are examples; (3) phenyl-propylamines compounds of which trimeton is an example, and (4) derivatives of pyridindene, of which thephorin is characteristic. All of these drugs have a common chemical configuration, a nucleus of the molecule, as it were, which is similar to that of histamine and epinephrine,



where X may be carbon, oxygen, or nitrogen, with various atomic groups attached.

The antihistaminic compounds are oily bases. Their salts are water soluble, and are rapidly absorbed from mucous membranes. While many are capable of parenteral administration without too great irritation, and while some are useful in aerosols, oral administration is satisfactory and preferable. The antihistaminics are excreted in the urine in 12 to 24 hours. Thus, in order to maintain effectiveness in the presence of a provocative agent releasing histamine, they should be administered about three times daily. The dosage varies from twenty-five to fifty milligrams for the older compounds, while for the more powerful phenergan and chloro compounds, five to ten milligrams is sufficient. Dosage should be adjusted to age, weight, and severity of symptoms.

While no significant chronic toxicity on prolonged administration has been reported for the antihistaminics, they all tend to have some untoward effects. Agranulocytosis may result from their continued use. They may cause drowsiness, dizziness, nausea, diarrhea, headache, muscular weakness, and general malaise. Individuals receiving the antihistaminics should be cautioned against driving automobiles on account of the possible drowsy and dizzy effects.

Wyngaarden and Seevers, in discussing the toxic effects of antihistamine drugs, find that severe toxicity from these compounds is rare, but that serious reactions occur from overdosage. While adults tend to show central nervous system depression, infants and children tend to develop convulsions.

It is difficult to understand how the Food and Drug Administration should have permitted over-the-counter sale of antihistaminics for the treatment of the common cold when there was so much evidence regarding their untoward action, particularly in causing drowsiness and dizziness, even on recommended dosage, and when there was so little indication that they have any value in the common cold. It is remarkable that another governmental agency, the Federal Trade Commission, should have been called upon to obtain agreement among American drug manufacturers to restrict claims for antihistaminics with respect to the common cold. The evidence now is clear that they have no value in preventing or curing the common cold, in spite of popular claims to the contrary.

In general the antihistaminics block the action of histamine, but not in all respects. It has been shown that some have actions similar to atropine, demerol, procaine, and quinidine (Dutta). Their antipyretic action appears to be related to their central depressant effect (Wood). They seem to be particularly useful clinically in skin allergies and in hay fever. They do not seem to be particularly helpful in chronic asthmatic conditions, smooth muscle allergy spasm, food allergies, or eczematous skin afflictions. Quantitative methods for comparing histamine antagonists in man have been devised by Bain.

Feinberg's clinical analysis of antihistaminics reports 60% to 80% improvement in seasonal hay fever. In chronic vasomotor-rhinitis, the incidence of relief is between 30% to 60% with these compounds. He concludes, however, that the typical asthmatic attack is influenced very little by the oral use of antihistaminic drugs. On the other hand, 60% to 80% of patients with allergic urticaria and angioneurotic edema respond well, with most consistent relief with regard to itching. Relief of itching in atopic dermatitis is a considerable factor in the improvement of the dermatitis since the tendency to scratching is removed. Pruritus is also considerably relieved by the antihistaminic drugs. Migraine headaches, however, are only occasionally relieved, and gastrointestinal allergy is improved in some instances only.

Feinberg concludes that the antihistaminic drugs are valuable adjuncts in the systematic treatment of allergic manifestations, but that they cannot replace more durable immunologic therapy of elimination and desensitization, nor can they supplant such an effective drug as epinephrine. It is advantageous to have considerable choice among antihistaminic drugs because of the marked differences in individual, clinical, and toxic response. It is Feinberg's opinion that, of all the assay methods tested so far, the antiwhealing action on the human skin shows greatest correlation with general clinical effectiveness.

The most comprehensive review of antihistamine agents in allergy was edited by F. F. Yonkman for the New York Academy of Sciences. This includes a valuable and stimulating discussion of the pharmacology of histamine by Sir Henry Dale, a thorough discussion of the significance of histamine in allergy, and then a detail consideration of various antihistamine agents, with a clinical summary.

Of serious import in dealing with antihistamine drugs is the multiplicity of confusing names used, many of them for the same chemical compound. Some have more than one tradename copyrighted by competing drug companies, with an N.N.R. name in addition, and further with other copyrighted names for over-the-counter sale and public advertising. The name situation in the case of antihistaminics reflects a ridiculous socio-medical predicament. Physicians will be wise to use only N.N.R. or pharmacopeial names for these drugs.

Currently one may find nineteen different chemical compounds available as antihistaminics, under fifty-four tradenames from thirty-nine companies.

Specific Desensitization in Allergy

A satisfactory but time-taking method of treating allergy consists in the identification of the provocative allergen by painstaking diagnostic tests, and then systematic desensitization using standard procedures. This assumes the ability of the patient to develop the specific antibodies required. Unfortunately, the results are not always as effective as desired either by the clinician or the patient. Mistakes may be made

in diagnosis, altered cellular stability may intervene during the diagnostic and therapy period, the patient may not have adequate globulin metabolism for antibody formation, and other factors may interfere. Specific desensitization can succeed in about 80% of patients with pollinosis (Vander Veer). Nonspecific protein desensitization had a considerable vogue a decade or more ago, and is still to be considered when other methods of treatment fail.

Summary

Effective treatment of allergy requires discriminating judgment and skill on the part of the physician. All aspects of the patient need to be considered in order to arrive at a judgment that will be helpful in deciding how best to proceed. Prevention of contact with provocative allergen depends upon identification by careful testing. Specific desensitization is the only effective cure.

The choice of drugs to be used in treating allergy depends upon many factors, all of which should be analyzed as carefully as possible by the physician. It is essential to do everything possible to promote the general physical well-being of the patient, with particular reference to cellular stabilization. Vitamin, hormone, mineral and protein balances should be fostered. Psychogenic factors should be controlled if possible. Infections should be removed by appropriate chemotherapy. For relief of the symptoms of allergy, a variety of drugs are helpful, including potassium iodide, the antipyretic analgesics, the aminophylline relaxants, the sympathomimetic compounds and local anesthetics, the parasympatholytic agents, and the antihistaminics.

Drugs should be used with careful regard for their possible effectiveness in the particular type of allergy confronting the physician. Certainly the marked enthusiasm for antihistaminics is hardly justified in the face of the confusing information about them. They constitute a valuable addition to the drugs available to physicians in the management of allergy, but the wise physician will not forget the many years of helpful experience with standard drugs on which no particular commercial pressure is being exerted.

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PROCEEDINGS OF THE TEXAS BRANCH OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

Fall Meeting, Austin, November 18, 1950

A Heat Labile Brucella Agglutinin Blocking Factor in Human Sera. V. T. Schuhardt, H. W. Woodfin, and K. C. Knolle, The Brucellosis Research Laboratory of the Clayton Foundation and the Department of Bacteriology, The University of Texas and the State Department of Health Laboratory, Austin, Texas.

The Relation Between the Calcium Content of Blood and Its Antitrypsin Action. Kenneth L. Burdon, Rufus K. Guthrie, Delores F. Rick, Department of Microbiology, Baylor College of Medicine.

Heat Resistance of Spores from Synthetic Media. O. B. Williams and O. F. Harper, Jr., Department of Bacteriology, The University of Texas, Austin.

Saturated Fatty Acids as Antisporulation Factors for Bacteria. W. H. Hardwick, Beverly M. Guirard, and J. W. Foster, Department of Bacteriology, The University of Texas, Austin.

Mutagenicity of Germinating Spores. R. B. Mefford, Jr., and Orville Wyss, Department of Bacteriology, The University of Texas, Austin.

Experimental Peritonitis in the Evaluation of Antibiotics. Russell F. Brame, Conan H. Millstein, and E. J. Pulaski, Surgical Research Unit, Brooke Army Hospital, Ft. Sam Houston, Texas.

In Vitro Sensitivities to Antibiotics of Gram Negative Bacilli Isolated from Children with Diarrhea. M. L. Beene, R. Gibbs, and A. F. Hansen, Department of Pediatrics, The University of Texas Medical Branch, Galveston.

Cultural Sensitivity Testing of Bacteria to Antibiotics. Conan H. Millstein and Anthony Contreras, Surgical Research Unit, Brooke Army Hospital, Ft. Sam Houston, Texas.

The Inactivation of Poliomyelitis Virus by Lyophilization. Morris Pollard, Department of Preventive Medicine, The University of Texas, Medical Branch, Galveston.

Additional Observations on the Distribution of Coxsackie Viruses. Margaret Neville Norris, Thelma D. Sullivan, and J. V. Irons, Texas State Department of Health, Austin.

Interference Between St. Louis Encephalitis Virus and Western Equine Encephalomyelitis Virus Along a Neuronal Pathway. Russell T. Jordan and Carl F. Duffy, University of Arkansas School of Medicine, Little Rock.

Suitable Media for the Growth of Mass Cultures of Pneumococcus. John B. Loefer and Russell G. Weichlein, Foundation of Applied Research, San Antonio.

Studies of Sulfur Metabolism of Brucella. L. J. Rode, C. E. Lankford, and V. T. Schuhardt, Department of Bacteriology, The University of Texas and the Brucellosis Research Laboratory of the Clayton Foundation, Austin.

Effects of Autoclaving Glucose and Phosphate on Growth Initiation. Hal H. Ramsey III and C. E. Lankford. Department of Bacteriology, The University of Texas, Austin.

Effect of Excess Vitamin Intake on "Normal" Bacilluria. Paul Ewing, Bernice Doucet, Randolph Clements, and G. A. Emerson, Department of Pharmacology, The University of Texas Medical Branch, Galveston.

Effects of Copalchi on the Glycolytic Systems of *Saccharomyces cerevisiae* and Mammalian Tissues. Joe B. Nash, William T. Moore, Joyce K. Howard, and Paul Ewing, Department of Pharmacology, The University of Texas Medical Branch, Galveston.

Abstracts

Three Unusual Fungus Infections in South Texas. F. W. Bieberdorf. Foundation of Applied Research, San Antonio.

The three cases presented are one each of the following: *Chromoblastomycosis*, *Sporotrichosis*, and *Maduromycosis*.

The chromoblastomycosis patient, a grain miller, age 54, had lesion develop just above the ankle. Gradually over a period of six months, the infection spread upward, covering most of his leg below the knee. Typical spores of *Hormodendrum* were observed in biopsy material removed from a lesion. Three months later, it had spread to other parts of the body.

The second case is a sporotrichosis of the knee and involving the bones of the knee. The skeletal form in which bones are involved is relatively rare. This is an unusual case—as to location, and as to duration. *Sporotrichum Schenkii* was isolated from the knee fluid of a man sixty-three years of age, who had a stiff knee for a period of over four years.

Knee fluid streaked on media developed colonies that exhibited a white, moist appearance instead of a brown, membranous growth. This yeast-like growth contained pyriform conidia intermingled with blastospores and on transfer, typical sporotrichum growth was exhibited. The original cultures remained in the yeast phase and on incubation of the knee fluid, it also developed fungus pellicles.

Monosporium apiospermum, the cause of madura foot or *Maduromycosis*, was observed in tissue removed in surgery and treated with 4% NaOH. The fungus hyphae with spores attached could be seen distinctly. On culture media, this rapidly growing fungus produces a white, cottony aerial mycelium which later turns gray or brown in color.

Evaluation of Mercurials as Antiseptics. Frank B. Engley, Jr. The University of Texas Medical Branch, Galveston.

A total of eleven representative inorganic and organic mercurial compounds commonly used as antiseptics were evaluated for bacteriostatic and bactericidal activity by means of *in vitro* and *in vivo* tests.

A combined *in vitro* and *in vivo* test with *Streptococcus pyogenes* (C203M) as the test organisms in mice was employed. The highly virulent streptococcus was exposed to aqueous mercurial preparations for ten minutes at 32–34C. The organisms were then inoculated into dextrose nutrient broth, thioglycollate broth, serum broth and injected intraperitoneally into mice. None of the mercurials tested was able to render the bacteria incapable of causing infection. The streptococcus was unable to grow in the dextrose broth due to the high bacteriostatic activity of the mercurials but grew out readily when sodium thioglycollate or serum was present. The tests in mice also indicated the relatively high level of toxicity of the mercurials.

By means of the paper disc and a micro-drop method of agar plate assay using sodium thioglycollate and serum as inhibitors, an evaluation was made of the bacteriostatic action of alcoholic and aqueous preparations of mercurials. These technics eliminated the influence of alcohol on the test organism (Staph. aureus #209). The results showed that none of the mercurials was bacteriostatic or bactericidal in the presence of sodium thioglycollate or serum although large zones of inhibition were noted on agar which did not contain chemical or natural inhibitors.

Commercial adhesive bandage preparations containing "Mercurochrome" were tested by the agar plate method. It

was demonstrated that in the presence of serum the mercurial was inactive. Some antibacterial activity was noted from the adhesive. Surface active agents of the various ionic types did not increase mercurial activity in the presence of thioglycollate or serum.

The Chick Embryo as a Test for Toxigenicity of Strains of Corynebacteria. Florence L. Evans. Department of Microbiology, Baylor University College of Medicine, Houston, Texas. Aided in part by grant from the Eli Lilly Co.

The present study was done with a reasonably large number of strains, many of which were recently isolated from cases, and the method developed gave reproducible results in embryonated eggs. The embryos killed following injection of cultures of *Coryn. diphtheriae* on the chorio-allantoic membrane showed marked hemorrhagic reactions in the embryo and congestion of the membrane, similar to that produced by toxin. It is unlikely that preformed toxin was present in detectible amounts in the doubly washed cultures. The injury produced, therefore, seems for the most part to be attributable to the toxin formed by the proliferation of bacteria. Toxigenicity, as determined by death of the embryos, correlated well with toxigenicity as determined by other more orthodox methods. Death of 80% or more of the embryos in test lots of 6 to 10 for each culture may be regarded as a specific indication of toxigenicity.

A Determination of the Temperature Characteristic of Spore Germination in a Putrefactive Anaerobe. Donald A. Mehl and E. Staten Wynne. Department of Plant Sciences, University of Oklahoma, Norman, and The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.

Through use of the general methods of Wynne and Foster (J. Bact. 55:61–68, 1948; J. Bact. 55:69–73, 1948), spore germination in putrefactive anaerobe no. 3679 was found to be apparently logarithmic at temperatures of approximately 20, 25, 30, 35, 40, and 45 C.

Rate constants of germination were determined at various temperatures, followed by substitution in the Arrhenius equation for the calculation of μ. A value of 10,300 calories was found for the temperature characteristic of spore germination.

The optimum temperature for germination appeared to lie between 40 C. and 45 C. At temperatures of approximately 20 C. germination was somewhat retarded, indicating the possible occurrence between 20 and 25 C. of a "critical temperature" in the sense of Crozier.

Rate of spore germination and rate of subsequent vegetative development were apparently affected differently by temperature changes.

The Hemagglutination Test as Applied to Tuberculosis. Leslie C. Murphy, Chief, Virology Section, Fourth Army Area Medical Laboratory, Brooke Army Medical Center, Fort Sam Houston.

The hemagglutination test, first described in 1948 by Middlebrook and Dubos, was applied to sera from known tubercular patients and bovines and to sera from cases unrelated to tuberculosis. Serial dilutions of sera absorbed twice with normal sheep erythrocytes are mixed with a suspension of sheep erythrocytes sensitized with a four times concentrated tuberculin (Lederle). After overnight incubation the hemagglutination reaction is read by gently tapping the tube and observing the size of the clumps. Patients' sera giving strongly positive syphilitic reactions, sera from acute streptococcal infections, and antisera prepared from many types of organisms do not produce a cross reaction. Paired sera on six individuals taken immediately before and thirty days after tuberculin skin testing did not produce a rise in titer. The tuberculin skin test is reported to increase the titer a few days after administration, but this effect is transitory.

Twenty-eight bovine sera from animals tuberculin skin tested one week prior to the test showed titers ranging from 1:4 to 1:256. Nine of these animals did not present gross tubercular lesions at slaughter. High titers were present in seven of these nine sera. There was no correlation between the presence or absence of gross lesions and the titer.

Titers of 1:32 and 1:64 have been obtained in patients who are tuberculin negative, chest X-ray negative, and without symptoms of the disease. Further work is in progress to determine if these are false positives or if tubercular infection is present which has not been diagnosed.

Laboratory-Acquired Infections in the United States. Robert M. Pike and S. Edward Sulkin. Southwestern Medical School of The University of Texas, Dallas.

A total of 1,342 infections presumably acquired as a result of laboratory work in the United States have been tabulated. Death resulted in 39 instances, a case fatality rate of 3.0 per cent. Approximately one-third of the infections have been recorded in the literature; the remainder were discovered by means of a questionnaire mailed to nearly 5,000 laboratories.

The laboratory-acquired infections include 775 bacterial, 265 viral, 200 rickettsial, 39 parasitic, and 63 due to fungi. At least 69 different agents were involved but brucellosis, tuberculosis, tularemia, typhoid fever and streptococcal infections accounted for 72 per cent of the bacterial infections and 31 per cent of all infections. The species of Brucella involved was stated in about 60 per cent of the 224 cases of brucellosis. *B. abortus*, *B. melitensis* and *B. suis* were about equally involved. Coccidioides out-numbers all other fungi as a cause of laboratory infection due undoubtedly to the highly infective nature of the chlamydospores. Laboratory infections with this agent are known to have occurred even in personnel who are not working directly with the fungus.

Trained scientific personnel were involved in 1,015 of the infections; the remainder occurred in students, animal caretakers, janitors, and others. Research accounted for 308 cases, diagnostic work for 455, production of biologics for 25 and classwork for 29, while in the remaining cases a combination of activities was involved.

The probable source of infection was indicated in all except 248 cases. The handling of clinical specimens and infected animals or ectoparasites accounted for 175 and 139 cases, respectively. Aerogenic transmission and "work with the agent"

were other frequently recognized sources of laboratory infection. There were 98 infections acquired in the autopsy room. Recognized accidents, excluding autopsy accidents, were involved in 215 instances. The type of accident responsible for the largest number of infections was associated with the use of a hypodermic needle and syringe.

A Comparative Study of Methods for the Isolation of *M. Tuberculosis*. Virginia D. Sanders. Fourth Army Area Medical Laboratory, Fort Sam Houston, Texas.

The paper is a report of the laboratory's findings on 214 specimens submitted for *M. tuberculosis* isolation. These specimens included 24-hour bladder urines, material obtained by gastric lavage, sputa, spinal fluids, catheterized urine from the pelvis of right and left kidneys, pleural fluids, bronchial washings, surface pus from wounds and pustules, and aspiration of abscesses.

The methods employed were culture in TB Broth (Difco), a modified version of Dubos' original broth, culture on Petragnani's medium, and inoculation of guinea pigs.

The concentration procedure used on those specimens likely to be contaminated (urines, sputa, gastric contents, and pus from various sources) was digestion with 3 per cent sodium hydroxide for 30 minutes, neutralization with 3 per cent hydrochloric acid, and centrifugation for 30 minutes; the supernatant discarded and the sediment mixed with 5 cc. sterile saline was used as the inoculum in all three methods. Penicillin in a dilution of 200 u/cc was added to the broth.

Of the three methods studied, the results showed the guinea pig the most sensitive with a yield of 25 positives against 17 positive Petragnani's cultures and 8 positive TB Broth cultures. In only eight instances were all three methods positive. In 9 cases Petragnani's method confirmed guinea pig inoculation whereas the TB Broth gave negative results. In eight instances both artificial media failed to reveal *M. tuberculosis* even though the specimens were positive by guinea pig inoculation. In no case in this series did TB Broth yield tubercle bacilli unless their presence was detectable also by Petragnani's method.

A GANGLIONIC PLEXUS IN THE HUMAN FALX CEREBRI

JOHN G. SINCLAIR*

The structure of the peripheral nervous system of man has been investigated in microscopic detail. Not much that is new is to be expected, and that only under unusual circumstances. The following account began with the casual examination of the cranium of an adult porpoise after the brain had been removed by Dr. Glenn Drager in 1949. The complete lack of olfactory nerves constituted an embryological problem. The area of dura covering what remained of the cribriform plate exhibited an extensive nerve plexus. This piece of dura was removed and sectioned serially in 1950.

The plexus proved to be centered on a pair of large stellate ganglia adjacent to a midline ridge equivalent to the falx cerebri of man. Sinclair (1950) gives a brief description of

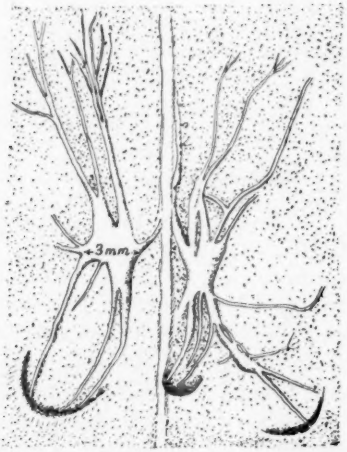

FIG. 1. Ganglia on the inner face of the dura of the adult porpoise adjacent to the midline falx cerebri. Folds in the dura occur at the junction of ethmoid and frontal bones.

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the ganglia. The ganglia are irregular and asymmetrical. The principal masses vary in width from 1½ to 3 mm. and extend 5 to 7 mm. in length. Figure 1 is a sketch of the pair lying on the inner face of the dura. Nerve cells average 18–20 microns in diameter. They are somewhat concentrated in the central masses but are also found along the course of all branches. The cell bodies are not smoothly rounded and some are elongate. They lie within defined capsules and are generally separated by fibers in various directions. Using nucleoli as critical landmarks for counting, 4,780 neurons were counted in the larger and 3,592 in the smaller ganglion. All branches to the dura accompany blood vessels. Several ventral branches extend into dural folds at the junction of ethmoid and frontal bones. They have not been followed in the adult animal.

Two porpoise fetuses were obtained from the Marine Laboratory of the Game, Fish and Oyster Commission at Rockport. The same ganglia have been seen under the binocular in these fetuses. In section, however, the cells of the ganglia are so small that they would not have been recognized prior to examination of the mature ganglia. This indicates their probable sympathetic nature. In serial sections of one fetus ventral rami from the ganglia can be traced between the frontal and ethmoid bones to the extensive venous plexus lining the blowhole. They have not been followed further.

It was at first thought that this plexus was connected to the terminal nerve complex but its position in man makes it very doubtful. In a four-months infant the whole cribriform area was sectioned longitudinally. The gross appearance of the block seen from both sides is shown in figure 2. A branch of the trigeminal nerve, the anterior ethmoidal, is seen skirting the lateral margin of the cribriform area toward the nasal fissure. The crista galli in the infant is a cresentic saddleback to which the falx cerebri is attached. Within the falx well above the crista is a plexus of nerves and ganglia. The plexus extends from the region of the nasal fissure back and well above the olfactory filaments and bulb.

A stretch preparation of the falx cerebri of a six-months human fetus was block-stained with hematoxylin. The surface was destained by means of a brush and acid. Figure 5 shows

the plexus. Ganglia are not sufficiently large at this stage to show much difference in caliber.

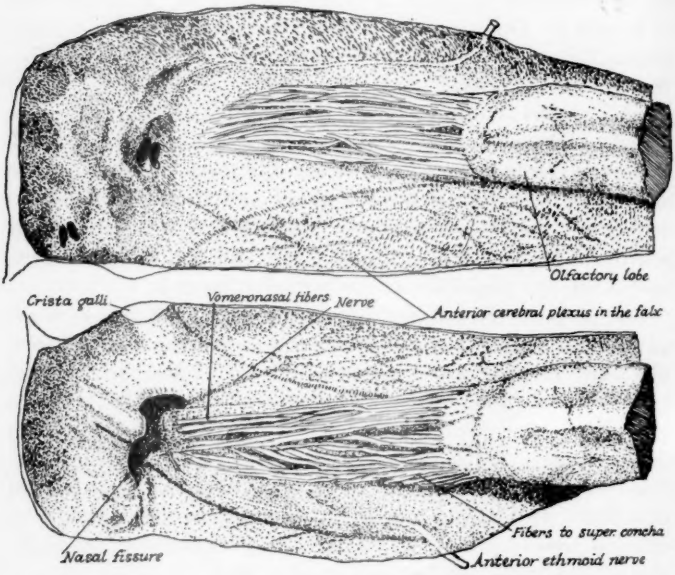

FIG. 2. Cribriform areas of an infant four months old as seen through a binocular microscope. The falx cerebri of each is tilted to show the relation of the nerve plexus, shown as a stained web, to the crista galli below it.

The microscopic picture of the ganglia in the falx of the four-months infant, pictured in figure 2, shows the plexus at various depths from the surface. It remains separate on the two sides except where the saddle of the crista is approached. Under low power the nervous tissue in a longitudinal section is drawn, figure 4, and illustrates the nerves branching across the midline with practically no collagenous tissue to indicate any separation. A detail of one cell cluster is given in figure 5. This sketch could serve equally well for the ganglia of the mature porpoise. The cells are 18–20 microns in diameter, without smooth outer boundaries. All are well encapsulated and are generally separated by diverse fibers though they may

occur in clusters or in rows. Approximately 850 ganglion cells have been counted on each side of the midline.

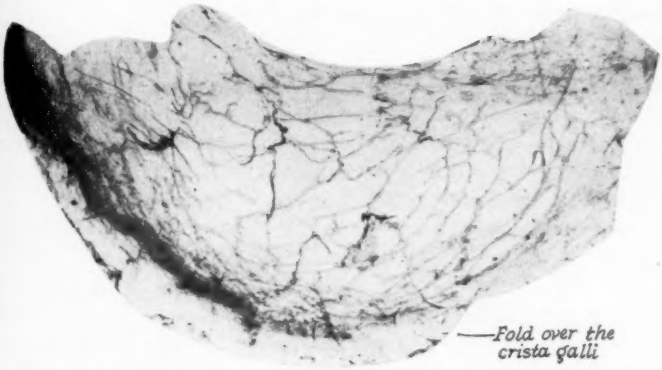

FIG. 3. Falx cerebri of a premature infant (sixth month) showing the ganglionated plexus above the crista galli. Stained by hematoxylin and the surface destained by brush and acid.

FIG. 4. Projection drawing of the falx cerebri from a four months old human infant taken just above the crista galli. The nerve plexus extends across the midplane and through the full thickness of the falx. Ganglionic clusters.

This whole plexus is dorsal to any terminal nerve fibers and may well be related to the anterior ethmoidal nerve instead. Many anatomy texts do not describe meningeal branches of the nerve. Larsell (in Morris, Human Anatomy, 10th edition, p. 1033) says, "It has been stated that branches are given from the nasal branch of the ophthalmic division to the dura mater of the anterior fossa." There is no specific reference and no mention of ganglia. In the rabbit there are definite intradural branches at the nasal fissure. These may be the source of the

fibers desribed as following the course of the anterior cerebral artery but ascribed to the terminal nerve. See Larsell (1918) figure 2. The anterior cerebral area may be innervated separately from the rest of the meninges.

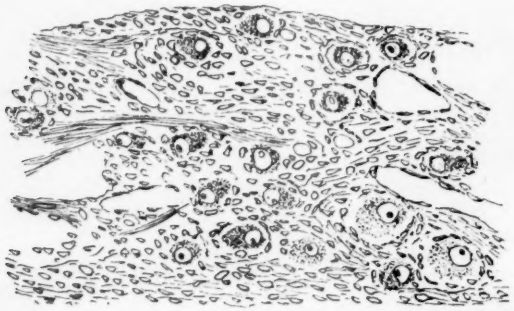

FIG. 5. High power detail of ganglionic cluster at point A in figure 4. Human falx cerebri from an infant four months old.

Further investigation of the connections of the plexus are in progress together with a re-investigation of the terminal plexus. The latter is far more complex than its size or functional importance indicate. Its present status is reviewed by Larsell (1950).

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A PLATE METHOD FOR DETERMINING FERMENTATION PATTERNS OF ENTERIC BACTERIA*

CARL L. SPAUR AND E. STATEN WYNNE†

Recently a number of tests have been devised to shorten and simplify bacteriological examination of material suspected of containing enteric pathogens. Thus, a regimen reported by Cook and Knox (1949) involved the use of a screening plate for rapid presumptive separation of pathogens and non-pathogens (Knox, 1949), a 1-hour urea test, and a 1-hour indole test. However, organisms tentatively identified as *Shigella* or *Salmonella* by this scheme were then inoculated into a set of 7 tubes containing various carbohydrates as a portion of the final identification procedure.

It was felt that a plate method of determining fermentation patterns of bacteria might result in a considerable saving of time and effort. This paper presents such a method, which represents essentially an adaptation of the principle of carbohydrate fermentation on plates as described by Knox (1949).

Experimental

The basal medium finally chosen had the following composition per liter: Difco proteose peptone, 10 gm.; Difco beef extract, 3 gm.; NaCl, 5 gm.; K_2HPO_4, 3 gm.; phenol red, 0.025 gm.; agar, 30 gm. The pH was adjusted to 7.6. After sterilization by autoclaving, the medium was cooled to 45° C. and 2 ml. of a sterile 10% solution of Difco sodium desoxycholate were added per 100 ml. Approximately 20 ml. were poured into ordinary Petri dishes. Strips of Whatman No. 3 filter paper approximately 60 by 3 mm. were sterilized separately in 50% solutions of appropriate carbohydrates (except for manitol, which was used in a 35% solution) and then dried in sterile Petri plates. Three such strips were placed in each dish

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of the melted medium and pushed to the bottom by means of sterile forceps. The strips were kept approximately parallel to one another and as widely separated as possible. The carbohydrate which a given strip contained was indicated by a code letter placed on the strip in pencil prior to sterilization.

After solidification the plates were dried at 37° C. for 18 hours prior to inoculation. As many as 4 broth cultures were tested per plate by means of line inocula at right angles to the paper strips. After inoculation, specially cut sterile sections of cover glasses measuring about 11 by 15 mm. were placed over the strip containing glucose. Incubation was for 24 hours at 37° C.

Fermentation of a given carbohydrate was indicated by a change in color of the medium from the initial salmon pink to canary yellow in the region near the strip containing the carbohydrate. The line of growth was surrounded by a small zone of precipitation. According to Leifson (1935) such zones are due to precipitation of desoxycholic acid at pH values of 6.5 or lower. The production of gas was shown by fissures in the agar underneath the cover glasses. Alkaline areas appeared pink to red.

The organisms used were obtained from the stock culture collection of the Department of Plant Sciences, University of Oklahoma. They were: *Aerobacter aerogenes*, *Aerobacter cloacae*, *Alcaligenes faecalis*, *Escherichia coli* var. *communis*, *Proteus mirabilis*, *Proteus morganii*, *Proteus* OX-19, *Salmonella choleraesuis*, *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella pullorum*, *Salmonella schottmuelleri*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Shigella paradysenteriae*, *Shigella sonnei*. Fermentation patterns of these 16 species of enteric bacteria have been determined by the plate method for glucose, sucrose, lactose, mannitol, xylose, and arabinose. Parallel determinations by the classical Durham tube method employing Difco phenol red broth base with 0.5% carbohydrate showed correlation in every one of the 96 reactions studied. It may be noted that the plate procedure requires a total of only 8 plates, while the classical method employs 96 tubes.

Summary

By use of the plate method described, fermentation patterns of enteric bacteria may be determined with considerably less time and effort than is required by the classical Durham tube method.

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BODY SALT AND WATER CHANGES DURING FRESH AND SEA WATER DROWNING*

H. G. Swann and Norma R. Spafford

During drowning there are great changes in the composition of the blood: in fresh water drowning, the blood usually is strongly diluted while in sea water drowning the blood becomes concentrated. Hemoglobin content (Brouardel and Vibert, 1880), red cell counts (Paltauf, 1892), Cl content (Inouye, 1906; Gettler, 1921; Yamakami, 1922) and other measurements (Moritz, 1944) have been used to establish these facts. A difference of composition of blood in the right and left heart is also usually noted, the blood being more dilute in the left heart in fresh water drowning and *vice versa* in sea water drowning (Moritz, 1944). This fact is used in forensic medicine to help determine the cause of death after submergence. We have recently studied the process of death from drowning in dogs, including the analysis consecutive measurements of the blood pressure, electrocardiogram, heart rate, heart sounds, venous pressure, breathing movements, plasma proteins, hemoglobin, lactic acid, pH and blood gases (Swann, Brucer, Moore, and Vezien, 1947; Swann and Brucer, 1949). It appeared from this study that enormous amounts of water enter the blood stream during fresh water drowning. This ran as high as 1 volume of water added to each volume of blood during 4 minutes of submersion. It was suggested (Swann, Brucer, Moore, and Vezien, 1947) that this influx, by upsetting the salt equilibrium of the blood, might in some way be responsible for the ventricular fibrillation that is characteristic of fresh water drowning (Banting, Hall, Jones, Leibel, and Lougheed, 1938; Lougheed, Jones, and Hall, 1939). In sea water drowning, not only does water leave the blood stream (Moritz, 1944;

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*From the Carter Physiology Laboratory, The University of Texas Medical Branch, Galveston. This paper reports a portion of the results obtained in a program of research on resuscitation. The program is sponsored by the U. S. Air Force Materiel Command, Wright Field, Ohio, under contract No. W 33-038 ac-14379. Two reports (Swann, 1949a,b) of limited distribution, have been made. The assistance of Miss Margaret Aldridge is gratefully acknowledged.

Swann, Brucer, Moore, and Vezien, 1947; Swann and Brucer, 1949), but salts appear to enter it, for Jetter and Moritz (1943) found large quantities of magnesium in the plasma of a dog drowned in sea water. Furthermore, Cot (1931) stressed the fact that large quantities of protein may be found in the lung fluids of drowning victims and therefore suggested that death from drowning may be caused as much by fulminating pulmonary edema as by aspiration of fluid.

These complex changes were reinvestigated in dogs, with particular attention to the movements of salt and fluid from the lungs to the blood and *vice versa*. In order to observe the movements of water, the tracer chemical, deuterium oxide, was employed.

Methods

The dogs were drowned as described in two previous reports (Swann, Brucer, Moore, and Vezien, 1947; Swann and Brucer, 1949) by putting a water-tight mask over their heads and then suddenly flooding the mask with either fresh or salt water. General anaesthesia was avoided in these experiments in order to have the animal in a condition as close to accidental drowning in man as possible. In its place local anaesthesia was used extensively.

During the course of the drowning, measurements were made of the femoral arterial blood pressure, heart rate, and intrathoracic pressure by optical methods as described previously. The latter measurement furnished quantitative information about the time and strength of the animals' breathing movements. The cannulas required for these measurements were inserted under local procain anaesthesia. Serial electrocardiograms (Lead II) were also obtained. In order to obtain information about the movements of fluid, a 3 ml. blood sample was drawn from the exposed femoral artery every minute during the course of the experiment. This was analyzed colorimetrically for its hemoglobin content and also for its deuterium oxide content. A 35 ml. sample of arterial blood was also drawn just before the start of the drowning and at 3 minutes after submergence; at post mortem a final blood sample was drawn from the left ventricle. These samples were

analyzed in duplicate for the following: whole blood oxygen and carbon dioxide content (Van Slyke and Neill, 1924), whole blood pH electrometrically at room temperature and then corrected to 38° by Rosenthal's formula (Rosenthal, 1948), serum sodium (Barber and Kolthoff, 1928), serum potassium (Harrison and Darrow, 1937), serum calcium (Clark and Collip, 1925), serum magnesium (Denis, 1922), serum chloride (Schales and Schales, 1941), serum sulfate (Letonoff and Reinhold, 1936), serum proteins and albumins by the micro-Kjeldahl method and the globulins computed by difference, and serum hemoglobin, where indicated, colorimetrically.

Immediately after the dogs had expired, the chest was opened and the post-mortem sample of blood was obtained from the left heart. Then a cannula was tied into the trachea just above its bifurcation and the cadaver held vertically to drain out of the lungs all possible fluid. The volumes of fluid drained were measured. The first 35 ml. to drain is called "Lung fluid sample A"; it is presumably mostly bronchial in origin. The last 35 ml. to drain is called "sample B"; it came deep from within the lungs and is presumed to be bronchiolar and even alveolar in origin. Both of these samples were analyzed for gases, salts, and proteins in the same fashion as the blood samples. After the lungs had been fully drained, they were dissected out and weighed. To this weight was added the weight of the fluid already drained from the lungs in order to obtain the total weight of the lungs of the just drowned dogs. Then by subtracting the weight of normal dog lungs, this being given by the ratio, lung weight/body weight in grams equals 0.0085 (Whittenberger and Kaye, 1947), the excess water in the lungs was estimated.

Deuterium oxide,[1] at a level of 10 vols. per cent, was added to distilled water to make the "fresh water" for the experimental drownings. In the sea water drownings, the D_2O was put into the dogs: 100 g., to which was added 0.9 g. NaCl, was injected intravenously some 20 minutes before the drowning was started. This brought the blood D_2O level of the dogs to

[1]A portion of the D_2O used in these experiments was furnished by Merck and Co., Rahway, New Jersey, through the courtesy of Mr. W. D. Nadler.

about 1.8 per cent. The D_2O centent of the blood and lung fluids was analyzed in a mass spectrograph.[2] In preparing for analysis, 3 ml. samples of blood or lung fluids were distilled *in vacuo* to dryness. The resultant distillate was sealed at once in glass ampoules until ready for analysis.

TABLE 1

Dog No. 1—Fresh Water Drowning

Minutes	0	1	2	3	4	5	Post mortem Blood	Lung fluid A	Lung fluid B
Blood pressure, mm. Hg									
systolic	192	*		100	43	..			
diastolic	96			38	26	24			
Heart rate/min.	92			40	24	†			
Breathing movements/min.	36		6	4	0	0			
Lowest intrathoracic pressures, mm. Hg	−12		−60	−88	..	..			
Blood									
Hemoglobin, g. %	14.8	14.6	14.3	10.4			5.9	0	.7
D_2O, g. %	0	.2	1.0	4.9			6.5	9.4	7.3
CO_2, vols. %								1.4	3.9
pH	7.33			7.24			6.76	6.28	7.47
Serum proteins, g. %									
albumin	4.8						.2	1.2	1.1
gobulin	1.7						.6	.2	.4
hemoglobin	0						5.6		
Serum salts, mE./L.									
Na	144						45	13	31
K	4.5						5.2	2.9	6.0
Ca	5.7						2.0	1.3	1.1
Mg	2.6						2.3	.6	1.4
Cl	91						38	14	30
SO_4	.4						.2	.1	.1

*In this and the succeeding tables, a blank space indicates that no measurements were obtained at this point.
†Ventricular fibrillation at 5:05.

In order to have a "sea water" of constant composition, an artificial sea water was made following the formula of Lyman and Fleming (1940). This solution has the same composition as open ocean water with respect to its main salts. It was made by preparing 23.477 g. NaCl, 4.981 g. $MgCl_2$, 3.917 g. Na_2SO_4, 1.102 g. $CaCl_2$, 0.664g. KCl, 0.192 g. $NaHCO_3$, 0.096 g. K Br, 0.26 g. H_3BO_3, 0.024 g. $SrCl_2$ and 0.003 g. NaF. These were dissolved in distilled water and the final volume brought

[2]Through the courtesy of the Humble Oil and Refining Co., Baytown, Texas.

to 1 liter. The concentration in milliequivalents per liter of each ion, the gas content and the pH, as ascertained by our own analyses, is given in Table 6.

Results

Five dogs were drowned in fresh water, the results for each dog being shown in Tables 1 through 5; 4 dogs were drowned in sea water, the results being presented in Tables 6 through 9. Table 10 shows miscellaneous data on all dogs: their weight, the time of last breathing movement, the weight of control lungs, the weight of the lungs after drowning, the amount of fluid drained from their lungs and the estimated excess fluid in their lungs at autopsy. Because the reactions of the dogs' blood pressures, breathing movements, blood gases and pHs followed the same general patterns as those described in the first series of dogs (Swann, Brucer, Moore, and Vezien, 1947;

TABLE 2

Dog No. 2—Fresh Water Drowning

Minutes	0	1	2	3	4	Post mortem Blood	Lung fluid A	Lung fluid B
Blood pressure, mm. Hg								
systolic	250			..	..			
diastolic	126			54	34			
Heart rate/min.	100	15	135	*				
Breathing movements/min.	18				2			
Lowest intrathoracic pressure, mm. Hg	–38				–53			
Blood								
Hemoglobin, g. %	17.7		11.5	8.3	7.2	7.9	0	0
D_2O, g. %	0	1.1	5.1	7.2		6.0	6.8	5.3
O_2, vols. %	20.9			1.3		.3		
CO_2, vols. %	42.0			27.4		24.1	7.1	7.4
pH	7.20						6.91	7.26
Serum proteins, g. %								
albumin	6.4			3.6		2.2	1.4	1.8
globulin	1.0			.9		1.1	.1	.6
hemoglobin	0			6.9		7.1		
Serum salts, mE./L.								
Na	149			56		44	13	25
K	3.7			3.6		4.8	3.2	4.7
Ca	6.3			1.9		2.0	.6	.8
Mg	2.3			2.7		1.8	.8	1.3
Cl	123			44		37	13	26
SO_4	.2			.1				

*Ventricular fibrillation at 2:13.

Swann and Brucer, 1949), no special comments will be made here upon the data on these variables.

A. Fresh Water Drowning

The first four dogs experienced ventricular fibrillation, this accident effectively terminating their lives, at the times shown in Tables 1–4. In all there occurred a fulminating anoxia and extreme acidosis at death. The same strong hemodilution took place as previously reported (Swann, Brucer, Moore, and Vezien, 1947; Swann and Brucer, 1949). Its course and extent may be followed from minute to minute by the change in hemoglobin it produced and by the appearance of D_2O in the dogs' bloods. Sometimes it occurred early, sometimes late; this is undoubtedly due to variations in the time at which the dogs first aspirated water. The hemodilution runs uniformly, potassium excepted, for the blood proteins and all the serum

TABLE 3

Dog No. 3—Fresh Water Drowning

Minutes	0	1	2	3	4	5	6	7	Post mortem Blood
Blood pressure, mm. Hg									
systolic	187	310	318	180	59	18	14	..	
diastolic	92	100	112	40	38	13	11	11	
Heart rate/min.	66	45	50	23	64	25	128	48*	
Breathing movements/min.	20		2	2	0	2	0		
Lowest intrathoracic pressure, mm. Hg	–21		–101	–71	..	–44	..	..	
Blood									
Hemoglobin, g. %	14.1	13.4	13.3	9.3	8.5	8.6			6.8
D_2O, g. %	0		.8	4.2		3.1			3.2
O_2, vols. %	16.7			5.0					4.1
CO_2, vols. %	60.8			45.9					41.5
pH	7.24			6.95					6.82
Serum proteins, g. %									
albumin	5.3			4.8					4.5
globulin	2.0			2.3					1.8
hemoglobin	0			1.5					3.8
Serum salts, mE./L.									
Na	140			93					77
K	7.4			5.8					8.3
Ca	5.4			3.1					2.5
Mg	2.5			2.0					.8
Cl	100			71					71
SO_4	.5			.04					

*Ventricular fibrillation at 7:37.

salts. The state of the dogs' bloods at death, as shown in the post morten blood sample, is nothing short of disastrous: serum Na diluted to 44 mE./L. (dog No. 2), serum Ca down to 2.0 mE./L., etc. The analyses of the fluid found in the lungs post mortem was done only in dogs No. 1 and 2, not enough fluid having been drained from the other dogs for the analysis. The lung fluids were found to be essentially a dilute serum. The first sample to drain (Sample A) had in it uniformly less salts than the last sample. Also the "deep" sample was relatively alkaline with respect to the blood. The presence of protein in considerable quantity shows that a pulmonary edema does in fact occur during drowning. The individual variation of the dogs was considerable; hence the record of each animal will be discussed.

Dog No. 1. This animal (Table 1) experienced ventricular fibrillation at 5 minutes, 5 seconds (5:05) after submersion. It is notable that ventricular standstill had taken place before

TABLE 4

Dog No. 4—Fresh Water Drowning

Minutes	0	1	2	3	4	5	Post mortem Blood	Post mortem Lung fluid
Blood pressure, mm. Hg								
systolic	169	300	292	203	41	48		
diastolic	80	102	119	102	26	28		
Heart rate/min.	66	32	43	57	48	28*		
Breathing movements/min.	15		9	7	1	4		
Lowest intrathoracic pressure, mm. Hg	–19		–75	–52	–29	–53		
Blood								
Hemoglobin, g. %	12.7	12.5	13.1	10			8.7	
D_2O, g. %	0			2.4			4.0	5.8
O_2, vols. %	15.8			9.0			.2	
CO_2, vols. %	46.9			40.4			35.0	
pH	7.29			7.04			6.86	
Serum proteins, g. %								
albumin	3.6			3.6			4.6	
globulin	2.5			1.6			1.5	
Serum salts, mE./L.								
Na	136			112			85	
K	4.9			7.9			9.3	
Ca	5.0			3.4			2.8	
Mg	1.5			.9			1.1	
Cl	105			79			67	

*Ventricular fibrillation at 5:17.

this, however, since the last arterial pulsations took place at 4:18. The serial hemoglobin measurements on this dog did not change appreciably until the third minute, suggesting that the dog did not absorb much water until just before 3 minutes. The D_2O analyses confirm this: the arterial blood D_2O abruptly increased from 1.0 per cent at 2 minutes to 4.9 per cent at 3 minutes. Because the dog was being drowned in 10 per cent D_2O, its circulating "blood" at this point was evidently 49 per cent drowning water and 51 per cent the dog's original blood. The dog's serum solutes underwent a great dilution: the post mortem heart serum contained 45 mE./L. of sodium—one-third its normal content. The other salts and proteins were similarly diluted. An exception is the serum K which increased slightly; the cause is to be found in the hemolysis that occurred; the *serum* hemoglobin increased to 5.6 g. per cent. The hemolysis undoubtedly released K from the red cells, and although

TABLE 5

Dog No. 5—Fresh Water Drowning and Asphyxia

Minutes	0	1	2	3	4	5	6	7	8	9	Post mortem Blood	Post mortem Lung fluid
Blood pressure, mm. Hg												
systolic	273	329	341	342	258	220	194	112	34	24		
diastolic	138	150	137	127	124	134	83	53	20	16		
Heart rate/min...	36	21	27	36	72	62	80	44	36	8		
Breathing movements/min. ...	6					5	5	8	0	0		
Lowest intrathoracic pressure mm. Hg	–24						–66	–65	..	..		
Blood												
Hemoglobin,g.%	16.5			16.3		15.4	14.9	14.7			14.8	
D_2O, g. %	0			.9							1.5	6.8
O_2, vols. %	17.9			1.5							.1	
CO_2, vols. % ...	45.8			55.8							44.7	
pH	7.23			7.12							6.70	
Serum proteins,g.%												
albumin	4.1			3.9							4.5	
globulin	3.4			3.4							3.0	
Serum salts,mE./L.												
Na				145							130	
K				7.5							10.9	
Ca	3.0			5.0							4.0	
Mg	.3			.4							.4	
Cl	123			122							109	

even this was being diluted by the incoming water, it was being formed faster than it was diluted, the net result being that serum K rose slightly. Anoxia also causes a rise in serum K (Fenn, 1940); this undoubtedly contributed to the observed change.

TABLE 6

Dog No. 6—Sea Water Drowning

Minutes	0	2	3	4	5	Post mortem Blood	Lung fluid A	Lung fluid B	Sea Water
Breathing movements/min.	20	7	7	7	7				
Lowest intrathoracic pressure, mm. Hg	−24	−34	−28	−29	−60				
Blood									
Hemoglobin, g. %	14.1	19.3	21.2	22.1	19.9	18.5	0	0	
D_2O, g. %	1.5		1.0			1.0	1.0	.9	
O_2, vols. %	14.7		1.5						.04
CO_2, vols. %	34.1		54.0						.75
pH	7.43		7.17				6.88	7.16	7.65
Serum proteins, g. %									
albumin	5.5		11.0			11.0	3.2	2.8	
globulin	1.6		2.4			1.8	.5	1.1	
Serum salts, mE./L.									
Na	151		198			209	274	276	488
K	2.7		3.3			3.0	6.1	5.5	10.1
Ca	7.1		12.1			13.4	12.9	12.9	19.8
Mg	2.1		13.7			33.3	74.5	79.1	110
Cl	121		128			269	321	329	537
SO_4	.5		.9			1.2	32.4	32.1	54.6

The fluids drained from the dog's lungs at autopsy showed a high salt and protein content. It is apparent that the blood salts must rapidly pass across the pulmonary capillary wall. Sample B (the last to be drained from the lungs) shows a higher salt content than Sample A (the first to be drained). Evidently, in spite of the churning effects of the breathing movements, the lung fluids were not thoroughly mixed. Furthermore, the post mortem blood had a higher salt content than Sample B of the lung fluid, so that it can be concluded that an equilibrium of these salts between the lung fluids and blood was not reached before the circulation failed.

The proteins found in the lung fluids were hemoglobin, albumin and globulin. The latter are no doubt responsible for the froth so often observed in the mouth and nostrils of

drowning victims. The hemoglobin would give this froth its characteristic pink tint. The fact that both albumin and globulin were both found in the lung fluid furnishes confirmation that a fulminating pulmonary edema did occur. One would not expect much protein and certainly not the large-sized globulin molecules in a simple diffusion fluid. Interestingly enough, the lung fluid proteins (excluding hemoglobin) were twice as concentrated as those in the terminal blood sample. Presumably the fulminating edema took place at a time when the serum proteins were high; then it must have ceased; but all the while large volumes of water continued to pass from lungs into the blood to dilute even further the remaining serum protein.

TABLE 7

Dog No. 7—Sea Water Drowning

Minutes	0	1	2	3	4	5	6	7	Post mortem Blood	Lung fluid A	Lung fluid B
Blood pressure, mm. Hg											
systolic	181	350	208	204	146	95	58	..			
diastolic	79	140	54	75	70	45	12	8			
Heart rate/min.	56	28	25	40	56	64	13	0			
Breathing movements /min.	6			11	9	0	0	0			
Lowest intrathoracic pressure, mm. Hg	−22			−100	−90	..	..	..			
Blood											
Hemoglobin, g. %	13.7	14.8	19.2	20.8		19.2			18.1	0	0
D_2O, g. %	1.7			1.4					1.2	1.1	1.2
O_2, vols. %	16.3			.2					.2		
CO_2, vols. %	35.7			42.9					27.5	16.2	
pH	7.28			6.92					6.53	7.07	7.76
Serum proteins, g. %											
albumin	3.6			7.3					6.2	2.1	2.0
globulin	3.6			5.0					4.7	.3	.6
Serum salts, mE./L.											
Na	157			230					245	277	259
K	5.6			6.9					7.8	9.6	9.6
Ca	5.1			10.8					12.5	11.3	12.3
Mg	1.9			32.7					58.6	69.4	78.1
Cl	116			216					269	313	332
SO_4	.4			1.3					1.7	27.8	28.2

The fact that the fluid last drained from the lungs contained 7.3 per cent D_2O and that the dog was drowned in 10 per cent D_2O shows that H_2O must have moved from the dog's body

into the drowning water. This is additional evidence for the occurrence of the fulminating pulmonary edema. As Table 10 shows, some 166 ml. of extra fluid were estimated as being present in the dog's lungs post morten. If we assume that the average content of D_2O in the lung fluid was 7.3 per cent, then 45 ml. of H_2O must have passed by pulmonary edema into 121 ml. of 10 per cent D_2O already in the alveoli to give a total volume of 166 ml. of 7.3 per cent D_2O. The drawback to this sort of estimate is that it assumes that only D_2O-free water moved into the lungs. But this assumption is not correct because as blood circulated in the dog, it picked up more and more D_2O. Hence, D_2O-containing edema fluid must have moved from the dog into the lung fluids. For this reason we cannot place much confidence in the estimate made above.

TABLE 8

Dog No. 8—Sea Water Drowning

Minutes	0	1	2	3	4	5	6	Post mortem Blood	Lung fluid A	Lung fluid B
Blood pressure, mm. Hg										
systolic	184	257	155	95	45	20	..			
diastolic	92	154	65	44	28	16	16			
Heart rate/min	88	29	49	86	68	52				
Breathing movements/min.				2	4	0	0			
Blood										
Hemoglobin, g. %	14.7	14.7	14.7	17.1	16.5			15.1	0	0
D_2O, g. %	1.8			1.5				1.4	1.2	1.3
O_2, vols. %	13.6			2.5						
CO_2, vols. %	36.4			49.9						
pH	7.30			7.08				6.84	7.15	7.12
Serum proteins, %										
albumin	5.0			6.8				6.5	1.2	1.2
globulin	1.9			4.8				3.9	.4	.3
Serum salts, mE./L										
Na	155			245				260	252	253
K	5.3			8.1				9.5	7.0	6.1
Ca	5.5			12.8				13.7	9.7	9.6
Mg	1.0			18.8				23.8	87.7	84.8
Cl	118			191				248	327	318
SO_4	.5			1.0				1.3	32.4	31.5

Dog No. 2. Ventricular fibrillation took place in this dog at 2:13, the earliest time after submergence for any dog in this and the preceding series. The dog continued making gasping movements for more than a minute afterwards, the last

gasp occurring at 3:44. The dog's blood stream was inundated with water between the first and the second minutes, as shown by the strong dilution of the blood hemoglobin and by the sharp increase in blood D_2O. At 2 minutes the dog's blood was apparently 51 per cent "drowning water" and at 3 minutes 72 per cent. The same extreme dilution of serum salts and proteins occurred, for they decreased to about one-third of the normal value in the post morten sample. Also a great hemolysis took place as shown by serum hemoglobins of about 7 per cent. Again, the serum K increased rather than decreased as did the other salts. Finally, as in dog No. 1, the lung fluids' D_2O content decreased and much serum protein was found in them, showing the occurrence of a fulminating pulmonary edema. Large quantities of salt also passed into the lung fluid. All of those events presumably took place within 2 minutes after submersion, for the dog's circulation failed with ventricular fibrillation at 2:13.

TABLE 9

Dog No. 9—Sea Water Drowning

Minutes	0	1	2	3	4	5	6	7	Post mortem Blood	Lung fluid A	Lung fluid B
Blood pressure, mm. Hg											
systolic	230	240	230	175	110	30	19	—			
diastolic	144	140	85	60	40	19	6	6			
Heart rate/min.	108	48	40	29	32	136	44	0			
Breathing movements /min.	5		5	5	4	0	0	0			
Lowest intrathoracic pressure, mm. Hg	−46		−80	−93	−116	..	..	..			
Blood											
Hemoglobin, g. %	14.4	18.7	20.6	23.9	22.3	22.8	22.8		21.9	0	0
D_2O, g. %	2.9			1.6					1.4	1.0	.9
O_2, vols. %	19.9			.1					.3		
CO_2, vols. %	36.4			53.3					41.9		21.0
pH	7.25			6.94					6.79	7.61	7.63
Serum proteins, g. %											
albumin	4.1			7.6					6.8	2.2	2.0
globulin	1.7			2.7					3.1	.3	.5
Serum salts, mE./L.											
Na	142			198					239	245	247
K	6.5			10.0					10.0	8.8	8.2
Ca	5.5			8.5					11.8	11.3	11.5
Mg	3.0			10.3					35.6	87.8	74.8
Cl	122			182					236	318	322
SO_4	.3			.4					1.6	35.3	26.2

Dogs No. 3 and 4. In both of these dogs, only small quantities of fluid could be drained from the lungs post mortem: 5 and 4 ml. respectively. Except for a D_2O analysis of the sample from dog No. 4, no analyses of these small volumes of fluid were made. In spite of the failure to drain, the lungs of both dogs apparently had some excess water in them, *i.e.*, 71 and 51 ml. Furthermore both dogs experienced strong dilution of blood hemoglobins, salts and proteins and both had a blood D_2O content at post mortem of about 4 per cent. Evidently large volumes of the drowning water were aspirated and then entered their blood streams. The heart fibrillated in one dog at 7:37, after circulatory standstill, and in the other at 5:17.

TABLE 10

Data on Drowned Dogs

Dog No.	Weight, Kg	Time of last gasp	Wt. of control lungs, g.	Wt. of lungs after drowning, g.	Volume of fluid drained, ml	Total excess fluid in lungs, g.
1	8.0	3:35	68	234	90	166
2	8.8	3:44	75	232	85	157
3	8.5	4:55	72	143	5	71
4	6.5	5:04	55	112	4	57
5	8.5	7:00	72	101	10	29
6	10.0	5:40	85	449	200	364
7	8.5	4:29	72	429	295	357
8	10.0	4:23	85	346	242	261
9	7.2	4:15	61	243	148	182

These data are of considerable interest because they show that drowning may occur with an accompanying violent hemodilution and with ventricular fibrillation and yet the lungs may be relatively "dry" at autopsy. The blood salts were roughly halved and yet at autopsy no frothy fluid could be drained from the bronchi and the lungs did not have the gelatinous water-logged appearance that characterized the lungs of dogs No. 1 and 2. What happened was apparently this: between the second and the third minute both dogs aspirated water into the lungs. This entered the blood stream explosively. But from that point on, no more water was aspirated.

Because the circulation was still adequate, much of the aspirated water was absorbed from the lungs and they were partially "dried up" during the 2 minutes of life remaining. In both dogs water was found in the upper trachea and mouth at autopsy, but evidently this was not aspirated. Tables 3 and 4 show that both dogs continued making strong inspiratory movements, for their intrathoracic pressures fell periodically until the fifth minute. But this apparently did not result in the inhalation of much water. Perhaps a glottic closure prevented the strong inspirations from aspirating water into the lungs. The rate of inspiratory movements in dog No. 3 was very slow; the inhibition of breathing was almost great enough to prevent aspiration of much fluid. On the other hand, the inspiratory rate of dog No. 4 was 4 to 8 per minute. And yet even this was not sufficient really to inundate the lungs with water.

Dog No. 5. This animal could not actually be described as a "submerged" dog. What happened was this: the one-inch rubber hose connecting the reservoir of water with the dog's head mask had trapped in it a large amount of air—a sort of air lock in the system. The air was never cleared out of the tube during the course of the drowning; the dog just rebreathed it back and forth, with only minimal quantities of water coming into contact with the dog's muzzle. The animal died therefore, more of obstructive asphyxia than of drowning. A small fraction of human drownings—Moritz (1944) guesses it to be "considerably less than 10 per cent"—are thought to aspirate no water after submergence and to die an asphytic death without much water in the lungs. The present experiment was technically faulty, but nevertheless it is the equivalent of this phenomenon of asphyctic death while submerged and so the data are included in the series. Only about 29 ml. of excess fluid were found in the dog's lungs at autopsy; the dilution of salts and hemoglobin was minimal. However, 1.5 per cent of D_2O was found in the dog's post mortem blood, showing that a little water did gain access to the animal. The process of death in this dog, with cardiac standstill at about 9 minutes and a strong metabolic and asphyctic acidosis, followed closely, as could be anticipated, the process of death which we

have described for simple obstructive asphyxia (Swann and Brucer, 1949).

B. Sea Water Drowning

In the four dogs (Tables 6, 7, 8, 9, and 10) drowned in sea water, results were far more uniform than during drowning in fresh water. The dogs' hearts ceased to function as effective pumps some 6–8 minutes after submersion. No ventricular fibrillations occurred, confirming our previous conclusion (Swann, Brucer, Moore, and Vezien, 1947; Swann and Brucer, 1949) that this accident does not take place in sea water drowning. The serial hemoglobin concentrations show that sudden hemoconcentrations occurred between 0 and 2 minutes in dog No. 6, between 1 and 2 minutes in dog No. 7, between 2 and 3 minutes in dog No. 8 and between 0 and 1 minute in dog No. 9. It is during these intervals that the dogs must have aspirated large volumes of sea water, which rapidly drew the blood water into it (Swann, Brucer, Moore, and Vezien, 1947). The violence and rapidity of this change is noteworthy: in dog No. 9, for example, the hemoglobin content before submergence was 14.4 g. per cent. One minute after submergence, it had changed to 18.7 g. per cent. Using the method previously developed (Swann, Brucer, Moore, and Vezien, 1947) to estimate how much water was lost from the blood, it appears that each ml. of the dog's blood lost 0.23 ml. of water in a single minute. The maximum hemoconcentration occurred in this dog at the third minute, with a hemoglobin content of 23.9 g. per cent. At this time, some 0.4 ml. of water had presumably been lost from each ml. of blood and had passed into the aspirated sea water. The D_2O changes confirm this, because at death much of the D_2O which was originally in the dog's body fluids exclusively, had passed into the sea water used for drowning.

But meanwhile, salts were passing rapidly in the reverse direction, for by 3 minutes of submergence the serum salt concentration in all dogs had increased greatly. In dog No. 8, for example, in which sea water was probably aspirated between the second and third minute, the serum magnesium increased 19-fold during that single minute. In the post mortem

blood samples of all dogs, the salt concentrations were very high. The single exception is the sulfate ion; this increased about 3-fold, but far less than would be predicted from its diffusion gradient (see below). In all 4 dogs the D_2O in the blood decreased during the drowning, this being very evident by the third minute of submergence. Apparently some D_2O-free water was entering the blood stream in these dogs.

The lungs of all 4 dogs were very water-logged. Table 10 shows that usually more fluid drained out of them and that the lungs had more total excess fluid in them than in fresh water drowning. Also, in sea water drowning a larger fraction of the total fluid in the lungs drained out by gravity. Thus, in dog No. 9, of 182 ml. excess fluid in the lungs, 148 ml. or 81 per cent drained, while in dog No. 1, of 166 ml. excess, only 90 ml. or 54 per cent drained.

The lung fluids were frothy and colorless. They lacked hemoglobin; no hemolysis of the blood occurred and hence dissolved extracellular hemoglobin was not available to tinge pink the lung fluids. The quantity of salts in the lung fluids was in each case roughly two-thirds that of sea water (see Table 6 for reference concentrations of sea water). This is testimony to the ingress of water from the blood into the lungs, causing a quick dilution of the sea water. The fact that the D_2O content of the lung fluids was almost the same as the content of the final blood sample shows how much body water moved into the pulmonary fluid. Moreover, the presence of 2–4 g. per cent of serum proteins in the lungs' fluid shows that a fulminating edema took place.

Discussion

The strong breathing movements of the dogs are indicated in the tables; periodic negative intrathoracic pressures of 60 mm. Hg. were common. These strong gasps undoubtedly aspirated fluid into the dogs' lungs. When animals are drowned in solutions containing tracer dyes, the dyes are found to penetrate deep into the lungs, even to "complete inundation of all the alveoli" (Martin, 1932; Moritz, 1944). This would bring the drowning solution, be it fresh or sea water, into intimate contact with the great surface of the aveoli. Then the rapid

exchange with the blood would occur, as we have shown, in a matter of seconds. Not only has the pulmonary epithelium a great capacity for absorption, as shown by Colin (1873), Winternitz (1920), etc., and as shown as well in these experiments, but also it apparently may secrete large quantities of edema fluid almost simultaneously.

During drowning the changes across the alveolar-capillary barrier may be summarized thus: in fresh water downing, the aspirated water, presumably because of the osmotic gradient, passes into the blood. But also a fulminating edema occurs, with protein-containing fluid passing into the lungs. Blood salts also pass into the lung fluid, by pulmonary edema and presumably diffusion as well. In sea water drowning, the sea water ions and H_2O pass into the blood stream. But meanwhile much body water passes out of the blood and into the alveoli. Also edema occurs, undoubtedly forcing water, salts and proteins out of the blood and into the alveoli. All of these changes start, it is apparent, in a matter of seconds after the dog aspirates fluid. The aspiration in turn depends upon how long the animals can hold their breath; some breathe before a minute has gone by, others (No. III, Swann and Brucer, 1949) not until 4 minutes have passed. But most breathe in for the first time at about 2 minutes, and the resultant violent change in blood composition, whether in fresh or sea water drowning, occurs within 3 minutes after submergence.

The changes in the blood resulting from drowning of either type are catastrophic. Particularly in fresh water drowning, where the serum salts are often reduced to about one-third cf their normal concentration, the change is so overwhelming that it seems impossible that the animal could long survive. Even in the two dogs which had relatively "dry" lungs on autopsy (Nos. 3 and 4), the blood salt dilution progressed to a point usually considered lethal, *i.e.*, serum sodiums of 77 and 85 mE./L. Conversely, in sea water drowning the serum Na exceeded 200 mE./L. in all dogs, this level of Na being thought incompatible with life (Fulton, 1946). In general terms, the more water found in the lungs post mortem, the greater were the changes in the blood.

There is a tendency for the lungs to be more water-logged in sea water drowning than in fresh water drowning. (But invariably, the lungs float at autopsy, Swann, Brucer, Moore, and Vezien, 1947). Taking all of the dogs studied in this and the preceding study, after sea water drowning an average of 244 ml. of excess fluid was found in the lungs, contrasted with an average of 175 ml. in the lungs after fresh water drowning. Furthermore, as shown above, the excess fluid tends to drain out more completely after sea water drowning. The reason for these two phenomena is to be found in the behavior of the aspirated fluid: in fresh water drowning, it passes primarily into the blood stream, while in sea water drowning it tends to stay in the lungs, because of its hypertonicity, and to augment its own volume by drawing the body water into itself. Nevertheless, in spite of these considerations, the differences between the volumes of fluid to be found at post mortem in the lungs are overshadowed by individual variation; some dogs die with much fluid in their lungs and some die with only a little, whether fresh or sea water drowning is involved. The range in sea water drowning is, in this and the preceding series, 79 to 364 ml.; the range in fresh water drowning is 57 to 326 ml.

Ventricular fibrillation occurred not at all in sea water drowning, but frequently in fresh water drowning; it took place in all 4 of the dogs tested in this series (No. 5 is excluded). Combining this datum with that previously collected (Swann, Brucer, Moore, and Vezien, 1947), the accident has taken place in 8 out of 10 dogs which have been drowned in fresh water. It may take place as soon as 2 minutes after submergence, and it may not take place until after cardiac standstill. It is probably, at least in part, a K fibrillation(Wiggers, 1930); the electrocardiograms are exactly similar to those shown by Nahum and Hoff (1939) in K poisoning. As previously explained, this increase is thought due to anoxia and to hemolysis with consequent release of intracellular K. Electrocardiograms similar to those of K poisoning are produced by the experimental hemolysis caused by injecting saponin (Wener, 1949).

The final K concentrations were not high; they ranged from 5 to 9 mE./L. But because the other serum salts decreased so

markedly, the ratio of K to the other cations increased greatly. Whereas in normal dogs the ratio K/Na is about .035, after drowning the ratio had increased to .113 in dog No. 1, to .109 in dog No. 2, to .108 in dog No. 3 and to .109 in dog No. 4. KCl, when rapidly injected, causes ventricular fibrillation. We may presume, from the reports of Winkler, Hoff, and Smith (1938, 1940), that the fibrillating level of K is roughly 15 mE./L. Its ratio to Na is then about .10, the same ratio as has been found in the dogs during drowning. We may postulate that this is a "fibrillating ratio" and that it was reached in each of the 4 dogs. Sometimes it was reached early, as in dog No. 2, sometimes late, as in dog No. 3; its time of occurrence depends on the time and degree of hemolysis which in turn depends on the time and quantity of aspiration of water. It is noteworthy that in sea water drowning the serum K may even be doubled, rising much higher than in fresh water drowning. But fibrillation does not occur because the "fibrillating ratio" is never exceeded. This whole situation is, of course, another aspect of Ringer's experiment: it is the relative, and not the absolute concentration, of the main serum cations that sets the pattern of irritability.

The events in sea water drowning merit closer examination: if body water exclusively passed out of the blood and into the aspirated sea water, it would effect a concentration of the blood salts like that observed. But because, as we have already shown, in fresh water drowning, the pulmonary capillary membrane is freely permeable to salts as well as water, this hypothesis appears untenable. Furthermore, if only water passed into the lungs, the serum Mg. would increase only slightly. But it increased, even at the third minute, from 3– to 19–fold. It is evident that the increase in serum salts is due to diffusion of the ions from the concentrated sea water into the dilute serum and that this is responsible for much of the change.

With respect to the sulfate ion, in spite of a high diffusion gradient (the sea water contains 55 mE./L. of SO_4), little SO_4 appeared in the blood. There are two possible causes for this: the alveolar-capillary barrier might be relatively impermeable to SO_4, or else any SO_4 getting into the blood stream might at

once be precipitated as $CaSO_4$ and hence not be found in the serum. But the latter appears improbable; since sea water, known to be supersaturated with respect to $CaSO_4$, still can hold in solution some 2.5 times as many mE./L. of SO_4 as it can Ca, one would expect, if all sea water salts passed into the blood rapidly, to find the terminal serum SO_4 at about 12 mE./L. But it was found to be only about 1.5 mE./L. Hence the alternative explanation for the behavior of SO_4 is more plausible: the alveolar-capillary barrier probably prevents SO_4 from entering the blood; it must be quite impermeable to the SO_4 ion. This is an unexpected finding, because most capillary walls are known to be quite permeable to the ion. Sulfates have been successfully used, for example, in studies of interstitial water, a technic which requires free passage of the ion in and out of the capillaries (Lavietes, Bourdillon, and Klinghoffer, 1936).

In the last 3 of the 4 dogs, the composition of the terminal blood sample was close to that of "deep" lung fluid sample (Sample B) with respect to Na, K and Ca. The blood had more CO_2, but less Mg, Cl and SO_4. And the water of the two fluids had evenly mixed, since the blood and lung-fluid D_2O contents were the same. Evidently, much $MgSO_4$ stays behind in the lung fluid; so also does much Cl, electrical neutrality being maintained by the bicarbonate remaining in the blood.

During sea water drowning, the D_2O in the dog's blood was rapidly diluted. The source of this D_2O-free H_2O is thought to be, at least in part, the aspirated sea water. Because each salt ion from the sea water is originally hydrated with H_2O and not D_2O, the passage of the hydrated ions into the blood would bring H_2O along with them. Each Na ion, for example, would carry 8 molecules of H_2O with it. This, then, would effectively dilute the blood D_2O.

The fact that in fresh water drowning the serum salts are found in the post mortem lung fluids suggests that they diffuse from the relatively hypertonic serum through the capillary walls and into the relatively hypotonic fresh water which has been aspirated into the lungs. The presence of proteins in the lung fluids however shows that pulmonary edema also

occurred. One might, in fact, ascribe to the edema the presence of salts in the lung fluids during fresh water drowning and the dilution of the sea water salts in the lung fluids that took place in sea water drowning. However, it will be noted that in fresh water drowning the dilution of protein in the 3 minute serum sample was less than the dilution of Na or Cl. Using dog No. 3 as an example, while the protein was only slightly diluted at 3 minutes, the Na dropped from 140 to 93 mE. The same process is apparently at work in dogs No. 2 and 4. This suggests that NaCl was rapidly diffusing out of the blood at this time while the protein was passing out much more slowly. Taking these data for what they are worth, therefore, they suggest that in fresh water drowning salts do diffuse out of the blood rapidly, and perhaps they diffuse out earlier than the onset of edema. The final concentration of salts found in the lung fluid is undoubtedly controlled by both processes, diffusion as well as edema. And in sea water drowning, both processes are also undoubtedly involved in creating the final composition of the lung fluids. Finally in neither type of drowning, can edema be held solely responsible for the fluids in the lungs post mortem, since in every case the lung fluids were found to be dilute with respect to one or the other serum component.

It is difficult to know from these experiments how much fluid entered the dogs in fresh water drowning. Because they swallow much water during the course of experimental drowning, weighing the animals post mortem would not give a good measurement of the water absorbed. (Even with the esophagus tied the violent passage of fluids occurs, showing that the route is via the lungs, Karpovich, 1933.) We may compute, however, how much water must be added to 10 per cent D_2O (the drowning fluid) to produce the terminal blood concentration of 6.5 per cent D_2O (dog No. 1). If we assume that the 8.0 Kg. dog had a blood volume of 660 ml., then its blood water would be about 530 ml. If to this were added 985 ml. of 10 per cent D_2O, it would reduce the D_2O concentration to 6.5 per cent. This estimate suggests that 985 ml. of water entered the dog's body fluids. But large amounts of the incoming water undoubtedly entered the interstitial spaces—the computation

should perhaps be made for this volume of water, rather than for the volume of blood water. Using the same methods, assuming the extracellular water comprises 25 per cent of the dog's weight, the volume of water entering the dog comes to 3.7 liters—almost one-half of the dog's weight. However, this estimate we know to be too high from another datum. The amount of 10 per cent D_2O used for the drowning was roughly measured. One liter was actually taken from the reservoir during the drowning; of this, roughly 230 ml. was spilled and a small amount—some 100 ml. as a guess—was found in the stomach. These rough measurements give a balance of 670 ml. in the dog's body. Of this, 166 ml. was in the dog's lungs, giving finally 504 ml. in the dog's body fluids. This does not agree at all with the estimates made above.

But all of these estimates are crude; the blood volume probably expands, the pulmonary edema rids the dog of some of its extra load of water, the liver rapidly removes a large portion of the extra load, and so on. It is for this reason that an accurate estimate of the total water entering the dog cannot be made from our data. But in spite of this failure, the hemodilution and the D_2O changes furnish evidence that a great hydremic plethora takes place in fresh water drowning.

We may make a better estimate of how much body water enters the lungs in sea water drowning, because the sulfate ion does not appear to leave the lungs in appreciable quantities. This furnishes the necessary clue. In dog No. 6, 364 ml. of extra fluid were found in the lungs post mortem. Because a concentration of 32 mE./L. of SO_4 was found both in the fluid which drained first and that which drained last from the lungs, we may assume with some degree of confidence that this concentration of SO_4 was reasonably uniform through all the excess fluid in the lungs. The ratio then of the final concentration, 32 mE., to the original concentration, 54.6, times 364 ml. gives the volume of sea water present in the lungs. This was 213 ml.: 213 ml. of sea water in dog No. 6 was diluted with 151 ml. of the body water to get the final volume in the lungs of 364 ml. and the final SO_4 concentration of 32 mE. Comparable figures for dogs No. 7 and 8 are: 183 ml. of sea water diluted with 174 ml. body water and 154 ml.

sea water diluted with 108 ml. body water, respectively. (A similar computation for dog No. 9 is not permissible since the composition in SO_4 of the post mortem lung fluids was not constant.) Body water entered, therefore, the aspirated sea water in the lungs to the extent of 108 to 174 ml. This is roughly one-fourth of the blood water. It is obvious that a dehydration of considerable magnitude takes place in sea water drowning. When it is also considered that relatively large amounts of salts enter the blood, it is apparent that in sea water drowning the animal's electrolyte equilibrium is profoundly disturbed.

All of these data invite reconsideration of the problems involved in resuscitation of the semi-drowned. These have been briefly described by us (Swann, 1949a) and will be more fully discussed by us in another paper. But two points should be made here. The first concerns the alterations in blood composition: they are spectacular and extreme in dogs; in man, in general terms, the same changes in blood chemistry take place (Moritz, 1944; Swann, Brucer, Moore, and Vezien, 1947). Obviously, after the semi-drowned have been resuscitated, attention should be given at once to rectifying the blood composition. Concentrated plasma, or indeed substitution transfusions, should be given to those who are half-drowned in fresh water. And dilution of the dehydration and salt plethora, experienced by those submerged in sea water, would be necessary. Persistent phlebotomy would probably be required to mitigate the fluid plethoras caused by both the drowning process and as well by the treatment process. The blood hemotocrit or hemoglobin content or Cl content would furnish quick indices as to how extensively and how long to continue treatment.

The second point concerns the ventricular fibrillation of fresh water drowning. In our opinion this accident probably occurs frequently during accidental drowning of men. As we have pointed out, ventricular fibrillation is probably caused by the changes in potassium following dilution of the blood with water. Taking the Cl concentration as an index of dilution, ventricular fibrillation occurred in all dogs whose serum Cl fell to 71 mE./L. or lower. This figure gives a clue to the

question as to how frequently ventricular fibrillation may be expected to occur in man. Moritz (Table 9, 8) summarizing the Cl content of the blood in fresh water drowning, shows 34 cases of human drowning; in 16 of these, the blood Cl (either whole blood or serum, and regardless of source) fell below 71 mE./L., the level which in our experience is accompanied with ventricular fibrillation. Ventricular fibrillation probably occurred in each of these 16 cases; they comprised about one-half of the total reported. The accident then appears to be frequent; perhaps half of human drownings in fresh water experience it. The accident is usually considered, outside of the laboratory or operating room, to be irremediable (Wegria, 1944). In drowning men, it may even occur, as it does in dogs, at 2 or 3 minutes after submersion, at a time when the victim is still making breathing movements. Its prevention depends, if our hypothesis is correct, upon rectification of the salt imbalance.

Summary

1. Measurements of the following were taken serially during the process of experimental drowning in dogs: blood pressure, breathing movements, electrocardiograms, blood gases, blood proteins and serum salts. Both fresh water and sea water drownings were investigated. At post mortem, the lung fluids were drained and analyzed for their salt and protein content. Deuterium oxide was employed to follow the changes in body water during the drownings.

2. During fresh water drowning, the animals' blood streams are inundated with water usually by the third minute after submergence. The "blood" at this time was often found to be half drowning water and half true blood. Dilution of the blood components reaches, after three minutes, to disastrous levels: serum Na down to 56 mE./L., serum Ca down to 2, etc. An exception is the serum K which rises slightly. This is thought due to anoxia and to the violent hemolysis that takes place; the serum hemoglobin content increases to 6–8 grams per cent. Ventricular fibrillation took place in all 4 dogs tested; at the earliest at 2 minutes and at the latest at 7½ minutes. In two cases, it took place after circulatory standstill.

The fluid draining from the lungs at autopsy, in fresh water drowning, was found to be a dilute serum. The fact that it contained serum proteins and that its original D_2O content was reduced is taken as evidence that a fulminating pulmonary edema occurred. The quantity of hemodilution taking place is roughly proportional to the amount of fluid found in the lungs at autopsy. In two dogs, however, although the blood was diluted with about one volume of water, the lungs were found to be relatively "dry" at autopsy. Much water entered the two dogs at about the third minute, but then entrance apparently ceased and the aspirated water was quickly absorbed from the lungs before the circulation failed.

3. During sea water drowning, the fluid aspirated into the lungs drew the blood water rapidly into it, resulting in a strong hemoconcentration. But the sea water ions, with the exception of sulfate, diffused in the reverse direction into the blood. At death the blood salt concentrations were extreme: Na at about 250 mE./L., Ca at 13 mE. and Mg at 30 mE. No ventricular fibrillations occurred. The lung fluid was found to be a diluted sea water containing about 2–4 g. per cent of serum proteins. Evidently a pulmonary edema occurred. About 150 ml. of the dogs' body water—some 15 ml./Kg—passed into the sea water aspirated into the lungs.

4. The changes observed in the two types of drowning may readily be explained by contrasting the tonicity of the drowning medium with that of blood. It is apparent that the pulmonary epithelium is exceedingly permeable, in either direction, to all ions except sulfate. In sea water drowning, salts diffuse in one direction while water is diffusing in the reverse direction. The pulmonary edema appears to take place simultaneously with the diffusion processes in both types of drowning.

5. It is suggested that the ventricular fibrillation characteristic of fresh water drowning is caused in part by the fact that, due to the hemolysis, the serum K remains constant while the concentration of other cations falls. This makes the ratio of K to the other ions high enough so that a potassium fibrillation takes place.

6. Drowning in men is briefly discussed, with consideration given to the violent changes in blood salts and to ventricular fibrillation.

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CALLING ATTENTION TO:

Items of Possible Interest to Our Friends

Muddled March, 1951

Clinical: L Amubree & Co discuss development of a hospital geriatric service (*Lancet* 1/123/Jan 20/51). R Chobot well reviews *Pediatric Allergy* (McGraw-Hill, NY, 51, 284pp, $4.5). ME Eisen & Co show high blood platelet adhesiveness in thromboangiitis obliterans (reduced by stopping cigarette smoking) & in thrombophlebitis (reduced by dicumerol) (*Circula* 3/271/51). CBJ Glass & Co. analyse thermal coagulation & iodoacetate index as detectors of abnormal serum proteins & underlying illness (*Proc Soc Exp Biol Med* 76/10/51). LS Kubie discusses the neurotic potential (*US Armed Forces Med J* 2/1/51). TC Lorme reports excess vitamin A as cause of food poisoning (*New Zealand Med J* 49/680/50). T McKeown & RG Record show high cold month incidence of anencephalic births (*Lancet* 1/192/Jan 27/51). JG Ramos & Co indicate mechanical (compression-expulsion) & chemical (dilating) factors influencing coronary flow (*Arch Inst Card Mex* 20/474, 534/50). HD Ruskin finds thiouracil cmpds useful in preventing paroxysmal cardiac arrhythmia (*Lancet* 1/134/Jan 20/51). G Sandor & JC Weill-Fage describe plasma euglobulin variations related to pathology of reticulo-endothelial tissue (*Presse Med* 59/53/Jan 17/51). GC Shattuck offers *Diseases of the Tropics* (Appleton, NY, 51, 783pp, $10). LJF Warnants considers treatment of cerebral palsy (*J Irish Med Asso* 28/35/51). E Wittkower & PG Edgell discuss psychosomatic aspects of eczema (*Arch Dermat Syph* 63/207/51).

Basical: L Brull & D Louis-Bar with mechanical heart show urine output varies with arterial pressure to 300 mm Hg, above which kidney capsule splits (*Arch Internat Physiol* 58/329/50). AC Burton analyses physical equilibrium of small blood vessels (*Am J Physiol* 164/319/51). EJ Delorme shows gradient loss of liver function in anoxia influences peripheral vascular responses (*Lancet* 1/259/Feb 3/51). HE & HC Harrison find calcium absorbed from duodenum 2–4 hrs after giving, vitamin D being helpful only if calcium is poorly soluble (*J Biol Chem* 188/83/51). G Klatskin & Co find no liver cirrhosis or fatty infiltration on prolonged alcohol ingestion if diet is adequate (*Yale J Biol Med* 23/317/51). F Latham discusses the oxygen paradox (*Lancet* 1/77/Jan 13/51). R Lorente de No's *Study of Nerve Physiology* is available in 2 vols (*Studies Rockefeller Inst Med Res* 131, 132, NY, $4). H Lundegardh offers evidence of salt transport by cytochrome enzyme system (*Nature* 167/71/Jan 13/51). A Ochoa analyses biological mechanisms of carboxylation & decarboxylation (*Physiol Rev* 31/56/51). RC Shukla finds acetylcholine exerts both stimulating & depressing effect on heart, both antagonized by calcium (*J Indian Med Asso* 19/317/50). CG Van Arman says adrenal epinephrin supply is increased by amino acid mixtures &

dihydroxyphenylalanine (*Am J Phsiol* 164/476/51). MW Woerdemar offers *Standard Atlas of Human Anatomy* (Scientific Publ Co, Amsterdam, 2 vols, 50, 1003 pp, 1154 illust, $18).

GENERALICAL: CM Anderson contemplates *Saints, Sinners & Psychiatry* (Lippincott, Phila, 51, 206pp, $3). Immunochemist WC Boyd offers *Genetics & the Races of Man* (Little Brown, Boston, 51, 453pp, $6). JB Conant amplifies *Science & Common Sense* (Yale Univ Press, New Haven, 51, 371pp, $4). E Glover questions *Freud Or Jung?* (Norton, NY, 50, 207pp, $3.5). CP Haskins writes provocatively *Of Societies of Men* (Norton, NY, 51, 282pp, $...5). K Horney analyses *Neurosis & Human Growth: The Struggle Toward Self-Realization* (Norton, NY, 51, 391pp, $3.75). JAM Meerlo describes the crime of mentocide (*Am J Psychiatry* 107/594/51). PM Morse & GE Kimball describe *Methods of Operations Research*—or how to help make decisions (Wiley, NY, with Technology Press, Mass Inst Tech, 51, 158pp, $1). A Pi Suner well crosses *The Bridge of Life: From Reality to Mystery* (Macmillan, NY, 51, 270pp, $3.75). S Radhackrishnan edits, translates & annotates *The Dhammapada*, the teachings of Buddha (Oxford Univ Press, NY, 51, 194pp, $2.5). FC Redlick & Co well discuss narcoanalysis & truth (*Am J Psychiatry* 107/568/51). DE Schneider offers *The Psychoanalyst & The Artist* (Farrer, Strau, NY, 50, 306pp, $4). RR Shaw well analyses communication through literature (*Unasylva* 4/105/50). FL Walker offers challenging blueprint for knowledge (*Sci Month* 72/90/51).

APRIL, 1951

REVIEWY: P Clarkson reviews treatment of burns, recommending exposure method (*Lancet* 1/460/Feb 24/51). WH Crosby surveys paroxysmal hemoglobinuria ABlood 6/270/51). ME DeBakey and A Ochsner review hepatic amebiasis (*Inter Abst Surg* 92/209/51). JD Feldman surveys endocrine control of adrenals (*Anat Rec* 109/41/51). AB Gutman analyses recent advances in uric acid metabolism & gout (*Bull NY Acad Med* 27/144/51). E Held analyses birth pain (*Schw Med Wchnschr* 81/227/Mch 10/51). W Huber analyses biological action of radiation (*Naturwiss* 38/21/51). AGE Pearse well reviews histochemical methods tributing pioneer FV Raspail (1794–1878) (*J Clin Path* 4/1/51). RJ Riley reviews pulmonary gas exchange (*Am J Med* 10/210/51). A Selzer reviews chronic cyanosis (*Ibid* p 334). H Sobotka surveys chemical tests for malignancy (*J Mt Sinai Hosp* 17/1021/51). N Thyresson reviews thallium joisoning & Studies its metabolism with Tl^{204} (*Acta Dermat Verner* 31/3/51). LB Turner surveys ballistocardiography (*J Mt Sinai Hosp* 17/1060/51). CJ Watson reviews porphyrin metabolism & porphyria (*Lancet* 1/539/Mch 10/51). PO Wolff in reviewing drug addiction calls for synthetic codeine substitute (*J Pharm Pharmacol* 3/1/51). CJ Wiggers notes incidents, accidents & advances in physiology from 1900 to 1920 (*Ann Rev Physiol* 13/1/51).

CLINICALLY: L Binet & MV Strunz artificial circulation by cardiac massage with artificial respiration of high oxygen for resuscitation after anoxia (*Presse Med* 59/121/Feb 3/51). KJ Franklin & Co describe inhibitory effects of severe asphyxia on kidney & urine flow (*J Physiol* 112/43/51). J Hamburger & G Mathe note shift in cellular water balance in anoxia (*Presse Med* 59/265/Mch 3/51). D Muting finds low serum methionine in liver disorder & high in nephrosis (*Ztschr Klin Med* 147/478/51). HL Osher & Co find khellin safe & effective in angina pectoris (*New Eng J Med* 244/315/Mch 1/51). Our EJ Poth & Co recommend neomycin for control of intestinal infection in abdominal surgery (*South Med J* 44/226/51). C quarti prefers electronarcosis to electroshock for psychiatric therapy (*Presse Med* 59/125/Feb 3/51). RT Lewis & Co describe Lewis blood group & show no salivary excretion of A substances in this group (*Med J Austral* 1/105/Jan 20/51). ML Thomson discusses sweating changes after UV radiation (*J Physiol* 112/22,31/51).

FUNDAMENTALLY: DW Adamson & Co describe analgesic action of butenylamines (*Nature* 167/153/Jan 27/51). HW Bansi discusses iodine metabolism in relation to thyroid & hypophysis (*Klin Wchnschr* 29/33/Jan 15/51). AWA Brown finds mosquitoes are attracted to warm moist dark bodies (*Nature* 167/202/Feb 3/51). A Berger writes *Medicinal Chemistry* (Interscience Publ, NY, 51, 1085pp, 2 vols, $20). B Campbell proposes reinterpretation of structure of cerebral cortex (*Anat Rec* 109/277/51). I Corman & Co suggest urethane affinity for nucleoproteins is related to its mutagenic action (*Cancer Res* 11/195/51). F Ellinger discusses histamine hypothesis on biological action of radiation (*Schw Med Wchnschr* 81/61/Jan 20/51). S Fiala describes naturally occurring inhibitor of blood coagulation (*Arch Internat Physiol* 58/386/51). V Friedberg notes permeability interference from antihistaminics (*Arzt Forsch* 5/29/Jan 25/51). RW Gerard shows abnormal neuron states in causalgia (*Anesth* 12/1/51). WE Heston finds nitrogen mustards are carcinogenic (*J Nat Cancer Inst* 11/415/51). BA Houssay & Co offer important *Human Physiology* (McGraw-Hill, NY, 51, 1100pp, $14.00). PA Nasmyth shows histamine reduces ascorbic acid content of adrenals (*J Physiol* VVB/BVE/51). G Perera summarizes adrenolescence in *Pituitary-Adrenal Function* (AAAS, Washington, 51, 220pp, $4). NW Shock prepares *A Classified Bibliography of Gerontology & Geriatrics* (Stanford Univ Press, 51, 597pp, $15). A Tennenbaum edits *Toxicology of Uranium* (McGraw-Hill, NY, 51, 334pp, $3). I Wesley notes mythelene inhibition of cobalt polycythemia (*Arch Internat Physiol* 58/412/51). PA Zahl edits *Blindness: Modern Approaches to the Unseen Environment* (Princeton Univ Press, 51, 576, $7.5).

GENERALLY: KJ Arrow discusses *Social Choice & Individual Values* (Wiley, NY, 51, 190pp, $2.5). JM Gibson eulogizes *Physician to the World: The Life of WC Gorgas* (Duke Univ. Press, Durham, NC, 51, 315pp, $4.5). L James observes *Americans in Glass Houses* (Schuman, NY, 51, 152 pp, $2). SX Radbill offers *Bibliography of Medical Ex Libris*

Literature (Hilpraind Press, Los Angeles, 51, 300pp, $4.5). SR Truman discusses *The Doctor* (Wms & Wilkins, Balt, 51, 150pp, $3). C Woodham-Smith biographizes *Florence Nightingale* (McGraw-Hill, NY, 51, 382pp, $4.5).

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ANALYTICAL: E Blickenstorfer reviews psychiatry & genealogy of acromegaly (*Arch Psych Neurol* 186/88/51). RM Calman & J Murray analyze transfer of antibodies across placenta (*Endeavor* 10/27/51). H Chaplin & Co review danger of vitamin D poisoning (*Am J Med Sci* 221/369/51) without reference to old notes (*Calif West Med* 44/149, 311-36). Nobelate WR Hess summarizes autonomic regulation (*Klin Wchnschr* 29/105/Feb 15/51). SS Kety reviews inert gas exchange in lungs & tissues (*Pharmacol Rev* 3/1/51). F Mietzsch surveys recent developments in chemotherapy (*Klin Wchnschr* 29/125/Feb 15/51). BM Patten analyses beginnings of embryonic circulation (*Am Scient* 39/225/51). Sir Sydney Smith summarizes history of forensic medicine (*Brit Med J* 1/599/Mch 24/51). O Vogt reviews localization of cerebral function. (*Klin Wchnschr* 29/111/Feb 15/51). H Weyl surveys 50 years of relativity theory (*Naturwiss* 38/73/51).

FUNDAMENTAL: From J Abajian to BW Zweifach, thousands of American experimental medicinalists report thousands of abstracts of millions of experimental facts beyond synthesis (*Fed Proc* 10/3–425/51). N Ambache shows cholinergic (contracting) & adrenergic (relaxing) ganglion cells in myenteric plexus (*Brit J Pharmacol* 6/51/51). AJ Barnett finds adrenergic stimulus to sweat glands with noradrenaline as post-ganglionic sympathetic transmitter (*Nature* 167/482/Mch 24/51). RK Bomrichsen & H Theorell offer enzymatic method for microestimation of ethanol (*Scand J Clin Lab Invest* 3/58/51). Appropriately WR Brain discusses cerebral basis of consciousness (*Proc Roy Soc Med* 44/37/51). H Braunsteiner finds mitachondria in blood cells (*Weiner Klin Wchnschr* 63/183/Mch 9/51). JB Cross finds Diasone & Promin effective in toxoplasmosis (*Proc Soc Exp Biol Med* 76/548/51). F Davies & Co describe cardiac nerve spirals (*Nature* 167/113/Jan 20/51). C de Duve & Co study permeability of mitachondria (*Nature* 167/389/Mch 10/51). H Ferner analyses histopathology of pancreas in diabetes. (*Arch Path Anat Physiol* 319/390/51). CA Handley & Co show diuretic action of thyroxin due to increase in number of functional nephrons (*J Pharmacol* 101/283/51). AV Hill, A Szent-Gyorgi & HH Weber sympose on muscle contraction (*Nature* 167/377–383/Mch 10/51). H Killian notes interference between cooling & narcosis (*Arch Klin Chir* 266/650/51). ME Latham & HW Elliott show codeine excreted unchanged by kidneys & intestines & some liver metabolism (*J Pharmacol* 101/259/51). L Laskowski & Co find alloxan bartonellosis (*Proc Soc Exp Biol Med* 76/475/51). J Lhermitte discusses visual hallucination of the self (*Brit Med J* 1/431/Mch 3/51).

G Meyniel & S Bazin show reduction in red cell charge after body irradiation (*Compt Rend Soc Biol* 144/1463/50). V Potter proposes fluoroacetate as inhibitor to study sequential blocking of metabolic pathways (*Proc Soc Exp Biol Med* 76/41/51). S Rapoport indicates over-ventilation in diseases of children aids dehydration (*Ann Paed* 176/137/51). JD Sabella & Co show locally applied vitamin A increases epidermal thickness (*Proc Soc Exp Biol Med* 76/499/51). M Wachstein & E Meisel show glycine reduces toxicity of mercury dieuretics (*Proc Soc Exp Biol Med* 76/523/51). BW Zweifach & Co propose tetrazolium staining for histochemical index of adrenal cortex activity (*ibid* p 446).

GENERAL: JAV Butler writes pithily on human organization & activity in *Man Is a Microcosm* (Macmillan, NY, 51, 162pp, $3). F Guerra discusses history of medicinal use of Thevetia Yocotl in Mexico (*Arch Inst Cardiol Mex* 20/686/50). MC Harriman offers *The Vicious Circle,* about the famed Algonquin Hotel round-table (Rinehart, NY, 51, 310pp, $3). Neutral Leslie James stones *Americans in Glass-houses* (Schuman, NY, 51, 152pp, $2). FV Kruse builds *The Foundation of Human Thought* in answering problem of science & ethics (Munksgaard, Copenhagen, 51, 404pp, $6). JE Moore evaluates public health control of syphilis (*Lancet* 1/699/Mch 31/51). A Scheinfeld offers *The New You & Heredity* (Lippincott, Phila, 51, 616pp, $5). G Woodcock & I Avakumovic make *A Biographical Study of Peter Kropotkin* (TV Boardman, London, 51, 463pp, 21s).